Studies in Plant Regeneration

By Elsie Kupfer

Owing to the great divergence of opinion as to the phenomena to be included under the head of "plant regeneration," the experimenter must consider, at the outset, which interpretation of the term he is willing to accept. In the narrowest sense — that held by Němec,* Pfeffer,† Prantl‡ and Frank§ — only those cases ought to be designated by this name in which the new parts formed after injury or loss exactly resemble in number and position the organs that have been removed. From this point of view, "true" regeneration in the higher plants, except for scattered instances, must be limited to the restoration of embryonic tissue in root and shoot. At the other extreme, the school consisting of Vöchting,‖ Goebel,¶ Klebs ** and Morgan,†† use the words to comprehend even the development of buds present on the part before injury. Under this latter aspect, therefore, regeneration is only a phase of normal vegetative growth.

Both of these interpretations seem to a certain extent open to criticism. The former definition would too sharply separate closely related occurrences. For example, to consider as a regeneration the new tip formed on the root of a seedling, as a result of cutting off one or two millimeters of its length,‡‡ and to exclude as such the roots formed from the stem of the same seedling upon the removal of the whole root, seems rather an arbitrary discrimination. Even in animals, where the reproduction of the organ in

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* **Nemec, B.** Studien über die Regeneration. 2 *seq.* 1905.

† **Pfeffer, W.** Physiology of Plants (trans. by A. Ewart), 2: 167.

‡ **Prantl, K.** Untersuchungen über die Regeneration des Vegetationspunktes an Angiospermenwurzeln. Arb. bot. Inst. Würzburg 1. 1874.

§ **Frank, A. B.** Die Krankheiten der Pflanzen. Zweite Auflage, 89. 1895.

‖ **Vöchting, H.** Über Organbildung im Pflanzenreich. Bonn 1878.

¶ **Goebel, K.** Organographie 42. 1900; also Über Regeneration im Pflanzenreich. Biol. Centralb. 22. 1902.

** **Klebs, G.** Wilkürliche Entwicklungsänderungen bei Pflanzen. Chap. V. 1902.

†† **Morgan, T. H.** Regeneration. 71. 1901. ‡‡ **Prantl, K.** *l. c.* 21.

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its original condition is the rule and not the exception, cases are known where the regeneration takes place at some distance from the wound, and at an angle to it, and yet the validity of the formation as a true regeneration is undoubted.* In any case where one or more organs are formed as a result of injury or loss, directly at the cut or at a distance from it, they should reasonably be considered as regenerated structures.

On the other hand, the word regeneration ought to be limited to those cases in which an organ is formed, *de novo*, at a place or under conditions in which it would not normally be found. It is in this respect that it seems best to draw the line between regeneration and ordinary growth and reproduction. Such an understanding of the process would exclude phenomena like the growth of latent root and shoot rudiments of cut willow twigs (which Vöchting, Morgan and Klebs place under the head of regeneration), the development of the shoot-meristems on separated leaves of *Bryophyllum* (which Goebel includes here), and the growth of the cotyledonary buds of the bean described by McCallum.† It is undoubtedly true, as each of these writers points out, that gradations between the two types of organ-formation exist, and that the conditions which bring about the development of a preformed bud may be very similar to those which occasion the generation of a new one. It has, furthermore, been proved that such development or formation may be caused without wounding. Nevertheless, it seems logical that, for an accurate understanding of the circumstances controlling organogeny, only such objects should be chosen for investigation as show at the beginning of the experiment no trace of the structure in question.

In the following experiments, therefore, an attempt has been made to obtain some further information as to the behavior of such budless pieces. As it is not always easy to determine positively whether or not primordia are present, there may be cases in the ensuing discussion where the distinction has been unintentionally overlooked; and there certainly are one or two instances where, for the sake of securing evidence on other points, the question as to the absence or presence of such buds has been purposely

* **Morgan, T. H.** Regeneration, 31.

† **McCallum, W. B.** Regeneration in Plants. Bot. Gaz. 40: 97. 1905.

disregarded. On the whole, however, the aim has been to separate such cases of pure regeneration from those which ought to be interpreted under the broader head of correlation.

The work was started at the propagating houses of the New York Botanical Garden three years ago, and has been in progress since. Unless a statement to the contrary is made, the experimental material which belonged almost exclusively to the higher plants, was placed in the cutting-frame in the experimental house and observed at short intervals. This frame is of the usual type, containing pure sand to a depth of about 6 inches, and covered by a partly whitewashed glass top. It may be added that in numerous instances the cuttings were tried, also, in other environments — saturated air, sphagnum, sawdust, charcoal, cotton, and water. But in all of these, the parts decayed with far greater rapidity than in the sand, and few positive results were obtained under other cultural conditions.

The writer desires to acknowledge indebtedness to Dr. D. T. MacDougal under whose direction and encouragement the work has been carried on, to Professor H. M. Richards for many helpful suggestions, and to Professor W. J. Gies for assistance on the chemical side of the experiments. Thanks are due also to the staff of the New York Botanical Garden, who have throughout provided every facility in their power for the successful completion of the work.

Various questions presented themselves for solution at the very outset of the work. To decide whether every budless part of the plant is equally capable of regeneration, the separate plant organs have been used as cuttings, and their behavior is described under the heads of *roots*, *stems*, *leaves*, *inflorescences*, and *fruits*. The question as to the rigidity of the polarity manifested by such pieces has been considered with the cases in which any irregularity has occurred. In a similar manner, the effect of external conditions has been treated both in connection with the special instances and as a general subject in the discussion following the experiments. With the object of ascertaining whether food is to be regarded as a necessary factor in regeneration, or whether the parts can, as has been stated, regenerate even under conditions of starvation, a further set of experiments has been conducted; and finally, existing theories as to the cause of the kind of organs produced in regeneration have been discussed in the light of these and other experiments with a view of determining their plausibility.

REGENERATION IN ROOTS

Roots afford excellent material for the study of regeneration because, while in many plants the roots normally produce buds, in a number of others, there is a marked tendency to form shoots only upon injury. Vöchting * showed conclusively that in cuttings of woody roots (poplar, *Paulownia*, elm, etc.), new roots are formed at the apical, and shoots at the basal (proximal) end of the part. The same polarity is normally manifested in root-pieces of *Taraxacum*, used by Goebel,† Wiesner,‡ and Küster || and of *Scorzonera hispanica* (Rechinger,§ Goebel ¶), though experiments have shown that this polarity could be partially reversed. For example, Wiesner succeeded in inducing shoot-formation from both ends of a *Taraxacum* root-cutting by a culture in light. Goebel demonstrated also that when the growth at the shoot-pole is arrested by a covering of sealing-wax or plaster, or when the callus is repeatedly cut off at this end, the distal end produces the shoots. It is noticeable, however, as Tittmann ** remarks, that the polarity is simply changed at one of the two poles; and that no case is known in which *both* poles produce the opposite type of organ from the normal.

In the work on roots several distinct types were used. Of these the horseradish (*Roripa Armoracia*) was chosen to begin with; this series of experiments was followed by one on the sweet potato (*Ipomoea Batatas*) and dahlia (*Dahlia variabilis*), as examples of thickened secondary roots; a number of fleshy tap-roots, carrots

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* Über Organbildung im Pflanzenreich 1: 84 seq.

† **Goebel, K.** Allgemeine Regenerationsprobleme: Flora 95: 400. Erganzungsband 1905.

‡ **Weisner, J.** Die Elementarstruktur und das Wachstum der lebender Substanz. 112. 1892.

§ **Küster, E.** Pathologische Pflanzenanatomie. 170. 1903.

|| **Rechinger, C.** Untersuchungen über die Grenzen der Theilbarkeit im Pflanzenreich: Abh. zool.-bot. Ges. Wien 43: 310. 1893.

¶ **Goebel, K.** Über Regeneration im Pflanzenreich. Biol. Centralb. 22: 1902.

** **Tittman, H.** Physiologische Untersuchungen uber Callusbildungen an Stecklingen holziger Gewächse. Jahrb. wiss. Bot. 27: 168. 1905.

(*Daucus Carota*), turnips (*Brassica Rapa*), salsify (*Tragopogon porrifolius*), radish (*Raphanus sativus*), and parsnip (*Pastinaca sativa*) were next used; and as an example of a woody root, *Pelargonium radulum* was selected. In these roots, attempts were first made to determine whether the part had any power to regenerate, then to solve the question of the tissues involved, and finally to secure evidence as to the action of external conditions and polarity in producing the structures.

Rechinger * used the root of the horseradish (*Roripa Armoracia*) to determine the minimal size of the piece capable of regeneration. The horseradish has on its surface, at the indentations left by the emergence of the secondary roots, little swellings. To these the name "buds" cannot be strictly applied, because, though the cells are rich in protoplasm, and evidently are "embryonic" in nature, there is no vegetative point as such present before growth begins. This develops, however, within a few days after planting the roots or using pieces as cuttings. The shoots thus formed, therefore, would not be classed under true regenerations, according to the definition. A transverse piece of the root, not more than 1.5 mm. in height was found by Rechinger to be capable of forming a new plant. As to longitudinal sections, he says, "Ist noch eine Sprossanlage vorhanden, so wird dies bald ausgebildet; sind nur das Cambium und die Gefässbündeln vorhanden, so kommt es über die Callusbildung nicht hinaus." He goes on to show, further, that when such slices pass through the rind, between the epidermis and the cambium ring — thus cutting the bundles which branch out to the buds — new shoots are formed at these intersections without previous callus formation.

In confirming * these results, some additional information as to the regeneration in the roots of horseradish was secured.

Experiment 1.— A large number of transverse pieces about 18 mm. in height were used as cuttings. Many of these, besides developing the buds already present on the rind, formed shoots *de novo*, from the cambium of the upper or lower surface indifferently, and occasionally from both. Usually when shoot-buds are already present on a cutting, their development precludes the production of others. Here, although in some cases such sections

* Rechinger, C. *l. c.* 322.

of 18 mm. had three healthy shoots already considerably advanced in development, they nevertheless gave rise to additional buds as outgrowths from the cambium (Fig. 1). Beyerinck * as well

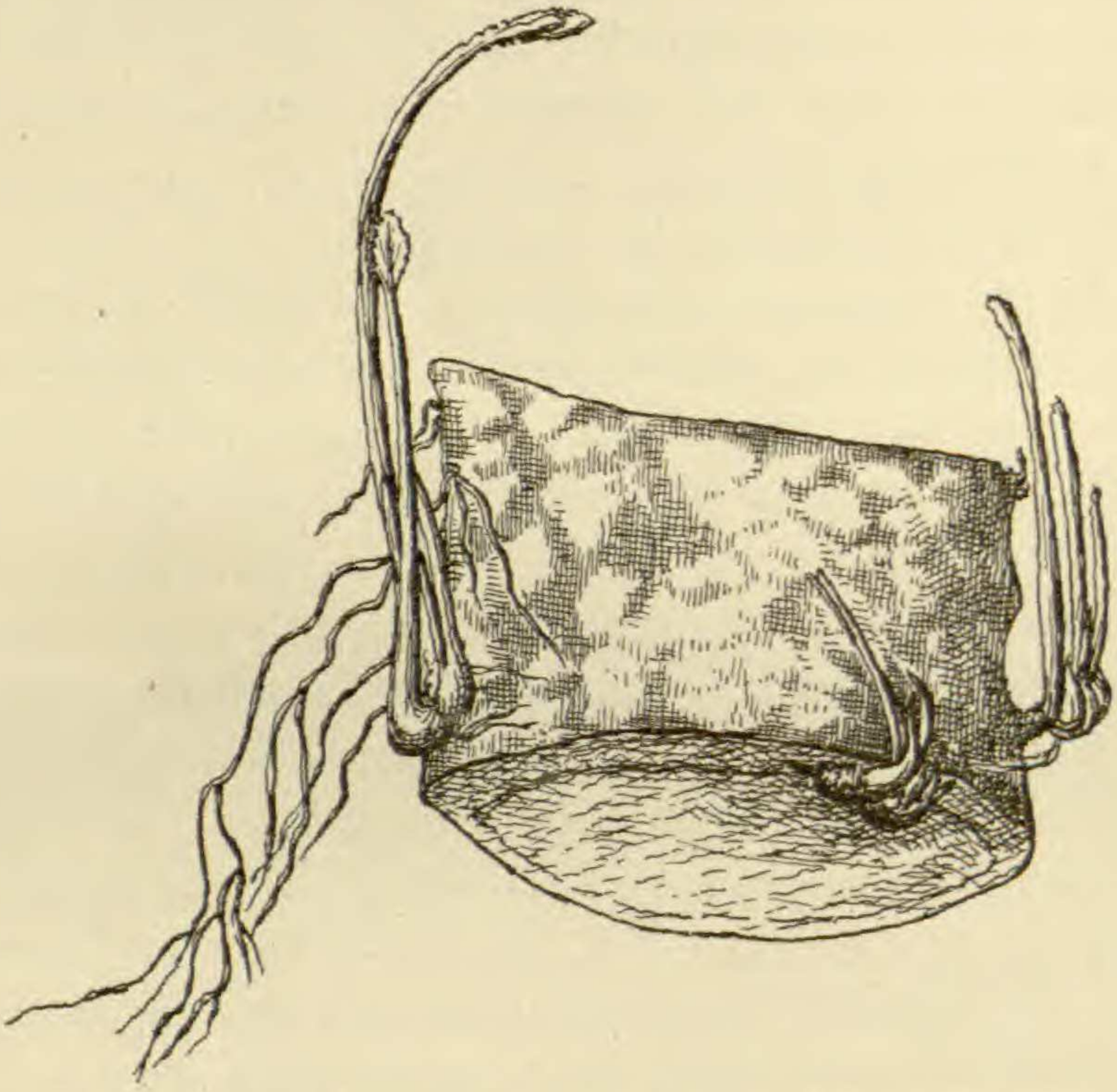

FIG. 1. Root-cutting of *Roripa Armoracia* showing a shoot regenerated from the cambium of the lower surface, besides lateral shoots developed from preformed buds on the rind.

as Rechinger stated that no regeneration takes place when the primary rind is cut away. The following experiment shows that this is not necessarily the case.

Experiment 2. — From a number of roots of horseradish, the rind, cortex, and cambium layer were removed. The part left, consisting of only the fundamental tissue and the bundles which are scattered through this, was cut up into slices averaging 20 mm. in height and 12 mm. in diameter. After four weeks shoots developed in the neighborhood of the scattered bundles, and more often at the distal end than at the proximal (Fig. 2). A callus of a few layers of cells preceded the formation of the shoot. In this case, therefore, the root can actually regenerate, in addi-

* **Beyerinck, M. W.** Beobachtungen und Betrachtungen über Wurzelknospen und Nebenwurzel, 52.

tion to developing the rudiments already formed. The power of forming the shoots lies in the cambium at the cut, and in the callus formed as a result of the injury, when no cambium layer is present. It will also be noticed that the tendency to produce shoots at the apical end of a horseradish root-cutting is at least as strongly marked as that operative at the basal end.

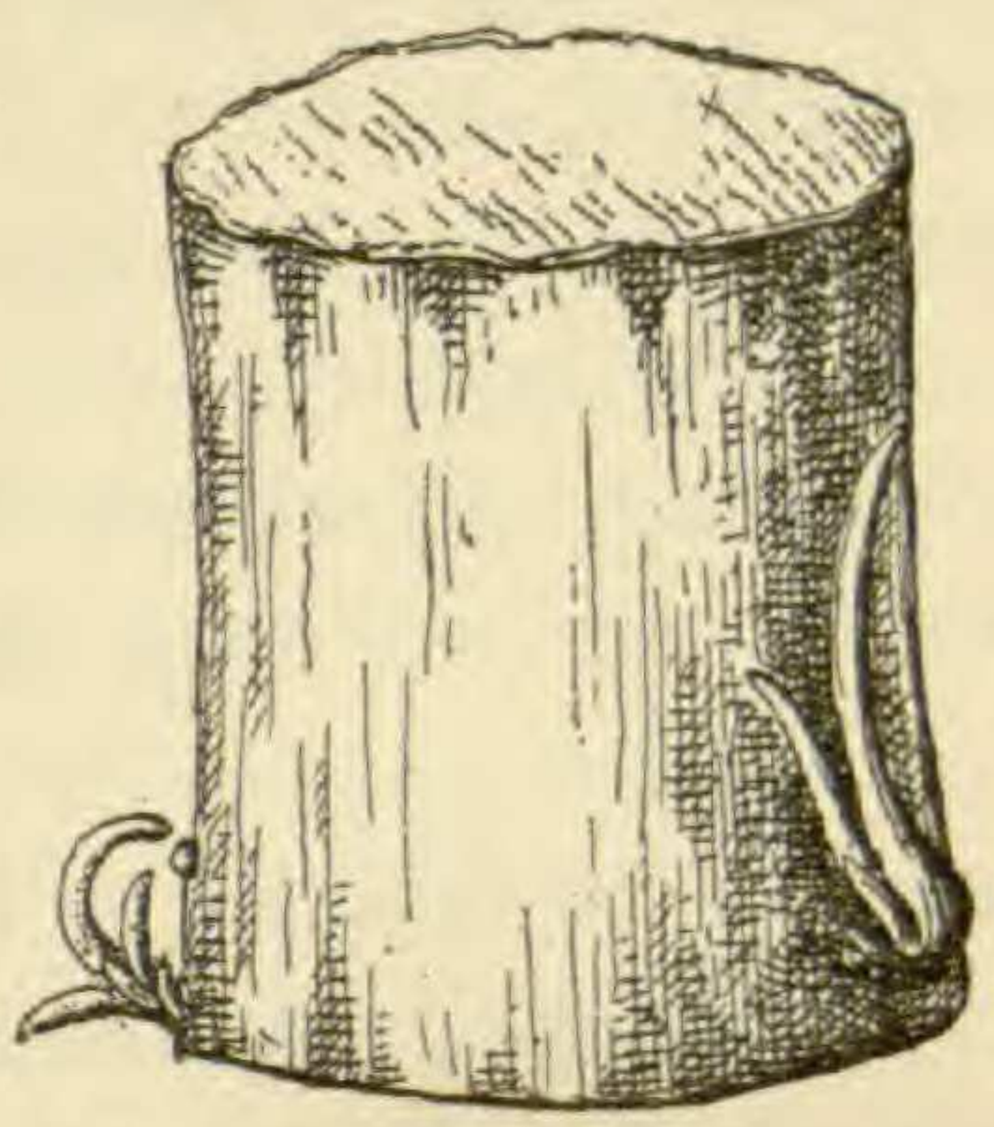

FIG. 2. Central tissues of root of *Roripa Armoracia*, from which the rind and cambium were cut away. Shoot-buds have appeared in several places.

In the sweet potato (*Ipomea Batatas*) and dahlia (*D. variabilis*) we have examples of thickened secondary roots which develop shoot buds between growing seasons, but care was taken that none should be present in the parts used in the experiments. The cuts made were similar to those described in the horseradish.

Experiment 3. —Transverse sections of sweet potatoes between 2 mm. and 3 mm. in height were placed underneath the level of the sand. Although a large number of these pieces grew moldy and decayed at once, a small proportion continued healthy and in 22 days produced roots. Many of these roots were true regenerations, inasmuch as they did not arise from the cambium and grow outward, after the manner of normal secondary roots, but appeared in the middle of both lower and upper cut surface. Microscopical examination showed that a callus of about four layers of cells had been formed on each surface whence the roots took their origin. The parts decayed without shoot-production.

Experiment 4. — The rind in which the buds appear was next removed from six of the tubers, and the pared parts were planted. Again roots were produced in numbers, but no shoots were formed in the four months before the cuttings decayed.

Experiment 5. — The converse of this experiment was also tried. Thin rind pieces, without visible buds and as far as possible freed from underlying tissues, were covered with sand. These also rooted within two weeks, but during the two months in which the parts remained intact, no shoots appeared. Therefore none of the parts of sweet potato in which shoot-buds were lacking ever regenerated such buds in these experiments.

Experiment 6.—Dahlia root-pieces cut in an exactly similar way have practically failed to regenerate. The transverse slices and the rind-pieces have developed lateral roots as outgrowths from the cambium. Parts from which the rind is lacking have not formed any organs. The production of shoot-buds from the rind is to be looked for, since it is a normal occurrence in the plant, but no instance has yet been observed.

As the next type of root, the fleshy tap roots of several plants were used; from these the leaf-bearing portion was removed, and the rest planted either as one piece or in sections.

Experiment 7. — A number of these which gave like results may first be mentioned together. Roots of carrots (*Daucus Carota*), turnips (*Brassica Rapa*), radishes (*Raphanus sativa*) and salsify (*Tragopogon porrifolius*) all produced secondary roots, as in the uninjured condition, but none gave rise to a shoot. The carrots and salsify formed calluses at the upper exposed ends, but although the carrots resisted decay for over five months, and the others for varying lesser periods, no further organ-formation took place.

Experiment 8. — Parsnips, however, afforded material for more extended experiments. Transverse sections of parsnips of various lengths were planted with the upper surface above the sand level. Calluses formed in two months' time on both ends. Shoots, it was found, could originate from the callus at either end; but, in the majority of cases, the apical end, underneath the sand, proved to be the surface active in their formation. In only six out of twenty cases did parts planted in the normal position regenerate shoots from the proximal end. One of these produced in addition a weak shoot from the apical end, and one from a cut made in removing a side branch which the root had possessed.

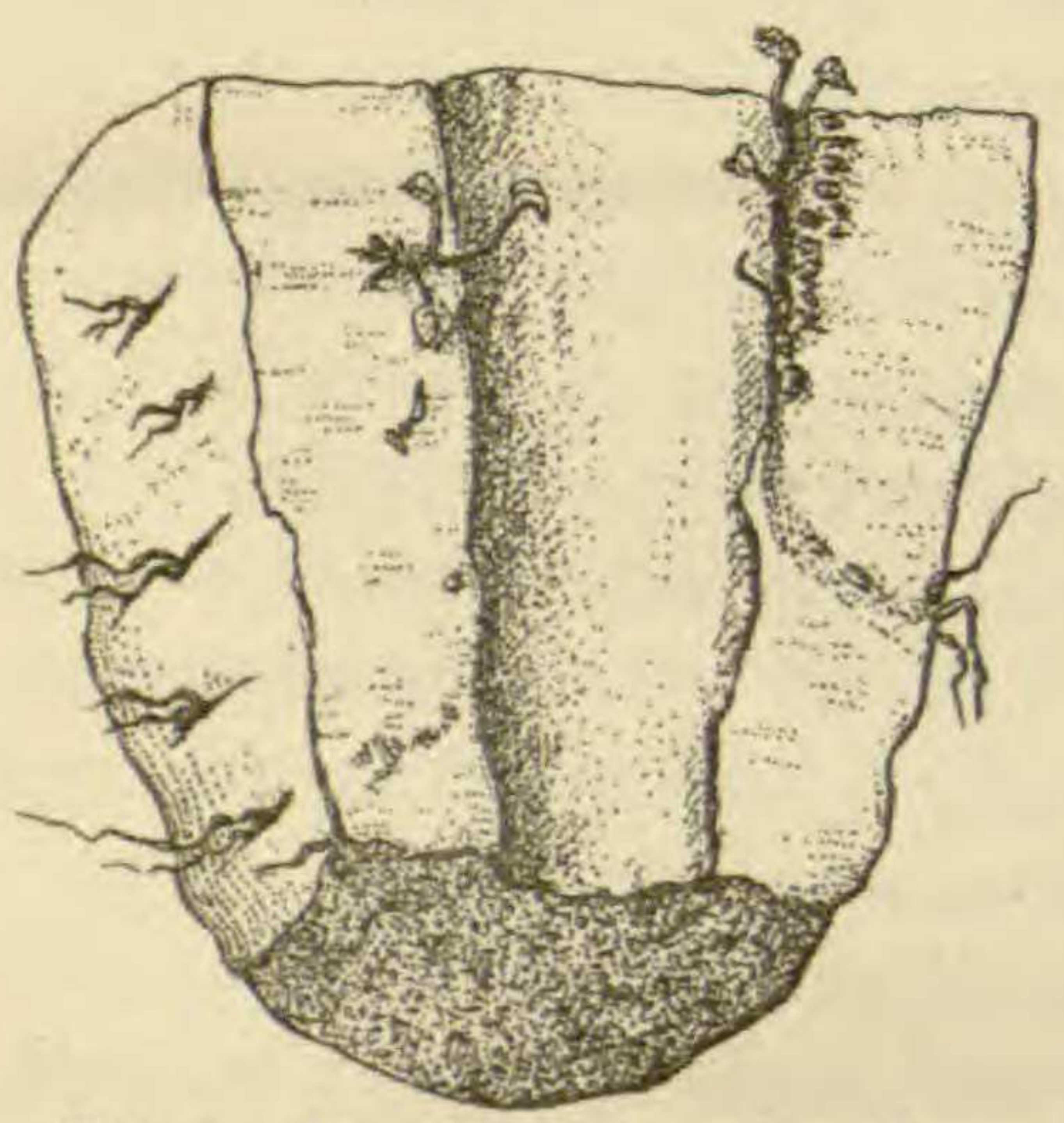

FIG. 3. Longitudinal section of root of *Pastinaca sativa*. Shoots have been regenerated along the cuts.

Experiment 9.—Similar parts were next planted in an inverted position. In this case, the shoots after 58 days all appeared on the basal surface — again the one under the sand.

Experiment 10. — Longitudinal sections of parsnips, laid in a horizontal position in the frame, developed shoots in the neighborhood of the cambium along both sides (Fig. 3). These pieces, therefore, resembled *Scorzonera hispanica*, as described by Goebel,* in forming shoots along the longitudinal edges. When placed vertically, the shoots originated only from the apical regions, as was seen to be the usual procedure in the transverse pieces.

Experiments 11 and 12. — The separate regions of the root were next isolated, as far as possible, in order to determine their capacity for independent regeneration. The rind alone, when used as a cutting, formed a callus on the lower surface towards the inside, from which shoots arose after 64 days (Fig. 4). Roots

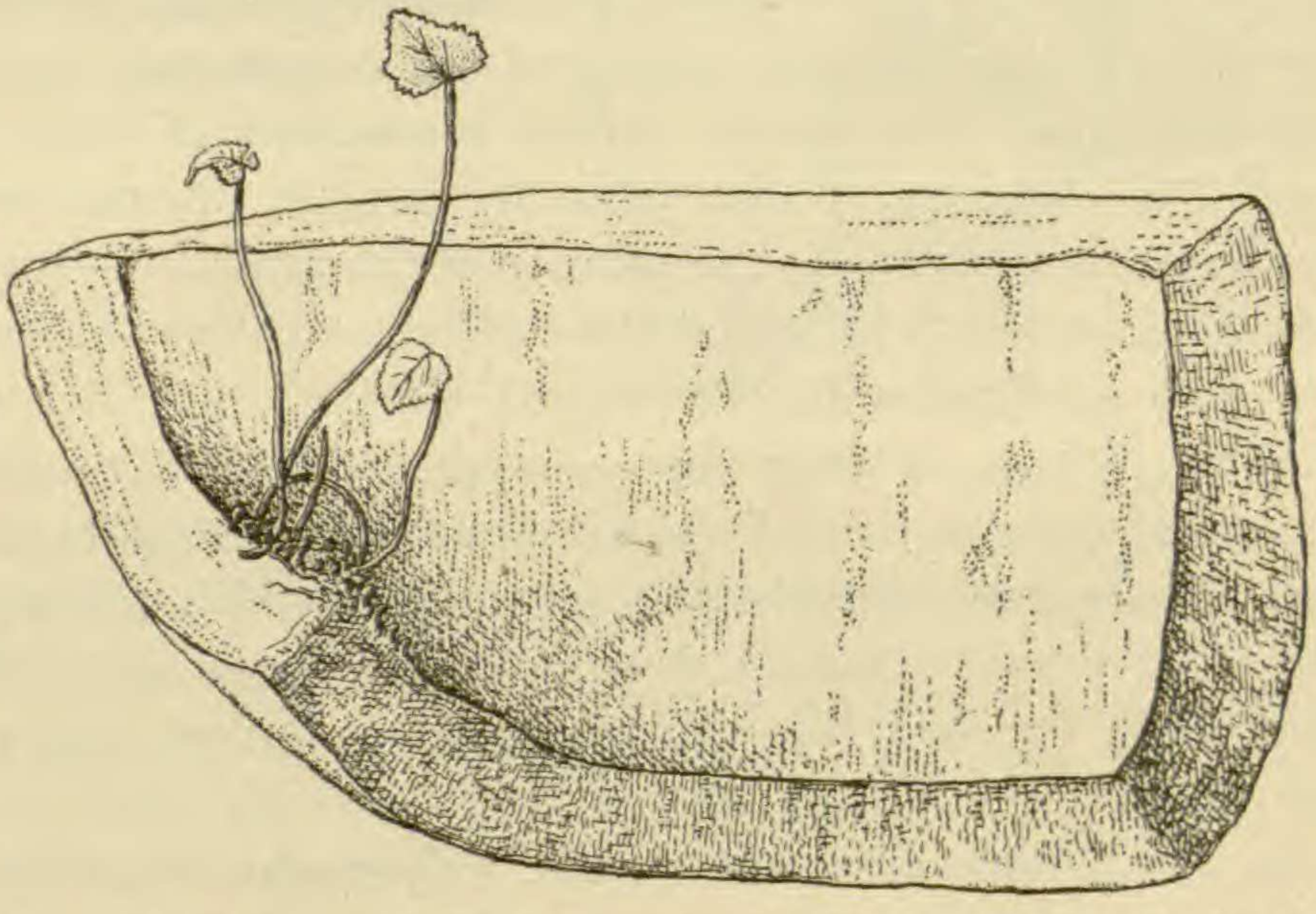

FIG. 4. Rind portion of *Pastinaca sativa* from the inside of which shoots have arisen.

grew from the outside as in the uninjured parsnips. When the central cylinder alone, also exclusive of the cambium, was planted, a callus again appeared at the apical end. From this in four weeks' time, several roots grew. No roots developed elsewhere in this piece, nor were shoots formed. Each region, therefore, is

* **Goebel, K.** Über Regeneration im Pflanzenreich. Biol. Centralb. **22**: 492. 1902.

capable of forming organs, but to a different extent. While the cortex can produce both kinds of structures — one as the result of regeneration and the other in the normal manner — the callus formed by the pith is able to originate roots only. This was the only case where roots formed from a callus in the parsnip.

The foregoing results indicate a close relation between shoot-formation in the parsnip and the action of some external factor, probably water. Vöchting has indicated that moist sand affords a complex of conditions, of which the water, and the contact with a hard substance are two. How the latter could prove active here is inconceivable. It is only for lack of any other evident cause, however, that one is willing to ascribe the difference in the behavior of the two surfaces to the greater amount of moisture in the sand. The air in a cutting-frame, while not saturated, is never dry, and amply suffices for shoot-formation in other instances. There are no other grounds, moreover, for considering water in liquid form as a usual factor in the production of these organs. The experiments that naturally suggest themselves as more definite approaches to the solution of the problem — such as growing the parts in a saturated atmosphere, or entirely below the sand, or in sterilized water, or covered on both sides by moist sphagnum, cotton, or filter paper — were tried without success. None of the parts so treated resisted decay long enough to regenerate. Pieces have recently been set up from which rectangular portions have been removed, thus giving an upper and a lower surface under the sand, but sufficient time has not yet elapsed to yield results.*

As an example of a woody root, *Pelargonium radulum* was chosen because of an accidental observation resulting from another experiment. A leaf-cutting had been made, which rooted freely and produced a shoot from the end of the petiole. The new shoot, in turn, formed a large root-system of its own. In removing the young plant from the sand, after it had been growing five months, its root was broken, part remaining in the frame. From this residual piece, three months later, a new shoot grew up, which turned out, upon investigation, to have arisen from the middle of the up-

* Beyerinck (*l. c.* 65) also found that the apical surface of this root seemed predisposed to form shoots. He offers no explanation, however.

per surface of the piece. Accordingly, an attempt was made to secure a repetition of the result through experiment.

Experiment 13.— The roots of several of these plants had forced their way through the openings at the bottom of the pots in which they were planted and had grown down into the gravel of the bench in the greenhouse. These were removed from the plants and used as cuttings. Up to the present time, in all which have regenerated, the procedure has been the same as that mentioned above. The shoots have all appeared from the middle of the pieces (Fig. 5). Specimens have recently been found in which some of these roots, while still on the plant, had sent up shoots. The gardener in charge says that this occasionally happens in this and other plants when the roots are "pot-bound."

FIG. 5. Root cutting of *Pelargonium radulum* which has regenerated a shoot from the middle of the piece.

In all the root-parts experimented with, secondary roots grew out rapidly, sometimes in a few days, or at most within a period of two weeks. These roots are not to be regarded as true regenerations inasmuch as their production is independent of injury. Nevertheless their almost universal occurrence serves to contest an inference reached by Vöchting in regard to both roots and shoots — that the different plant parts give rise to unlike structures with considerably more ease than to like structures. The results in root-cuttings and, as will be seen presently, in other plant parts as well, point to a different conclusion. The establishment of roots, both at places in which they normally occur, and as "adventitious" organs is a far more general and rapid phenomenon than the replacement of shoots. Less than half the kinds of roots used — and care was taken that all should be rich in a supply of food for expenditure in organogeny — formed any shoots; and in those which did, the appearance of roots antedated that of shoots by a period varying from two weeks to five months. This fact will be referred to later on.

Another point that deserves attention here is the lack of a rigid polarity in the root-parts mentioned. In none of the species in which shoots were acquired anew were these restricted to the basal end. In the horseradish the cambium of either or both surfaces proved active; in the parsnip the end in contact with the moist sand seemed commonly more effective in regenerating shoots, without regard to gravity; and in the *Pelargonium* these arose from the middle of the root. Furthermore, in one of the two cases in which roots were in the narrower sense regenerated, that is, in the thin transverse sections of the sweet potato, these developed from both sides. The evidence, therefore, seems to warrant the conclusion that polarity evinced by root-pieces without buds is less fixed than is generally believed.

REGENERATION IN STEMS

With the exception of internodes, parts of stems from which buds are absent have been little used in studies in regeneration. This may be because investigators have concerned themselves almost exclusively with the question of root-development on stems. In the stumps of some trees, though, the power to produce shoots from a callus derived from the cambium is a phenomenon very well known but not readily subjected to experiment. Nevertheless, some stems afford abundant scope for experimentation as to shoot-production. Experiments have been carried on with dicotyledonous stems, modified stems of various types, monocotyledons and a conifer. In the dicotyledonous stems, of which *Muhlenbeckia platyclados* and *Phyllocactus* (sp?) were taken as one type, aerial shoots of potato as a second, and the potato-tuber as a third, the first point upon which information was sought, was the behavior of stem-pieces of considerable size from which the buds had been cut. A comparison of such regeneration with cases in which one or more buds had been left was also instituted; and finally the regeneration of the internode alone was investigated.

Muhlenbeckia platyclados is well suited for the first purpose because of the ease with which the regions concerned in bud-production can be removed. The stem is a flat jointed organ without functional leaves in the adult condition, but with scales occurring in two orthostichies at alternating edges of the septa. From these regions roots appear when parts of the stem are planted. There are, however, no "root buds" in the sense in which the words are used for willow and poplar twigs.

Experiment 14. — Oblique cuts were made above and below the scale-bearing portions of the nodes so as to excise these together with any rudiments which might be present. The stems were then cut into pieces on October 29, 1905, and planted. On December 31, all had formed a slight callus on the basal cuts and roots had grown out from these. In one case a wound had been made in the lowest internode, and also a longitudinal slit just above the first node. From both of these regions additional roots appeared. Cuttings of this sort remained alive and healthy for over sixteen months and produced a richly-branched root-system, but no new shoots (Fig. 6). During this time no change in form or secondary thickening of any kind took place. This is all the more unusual, in that, as will be seen later, when a single bud was left on the piece, such thickening was plainly visible.

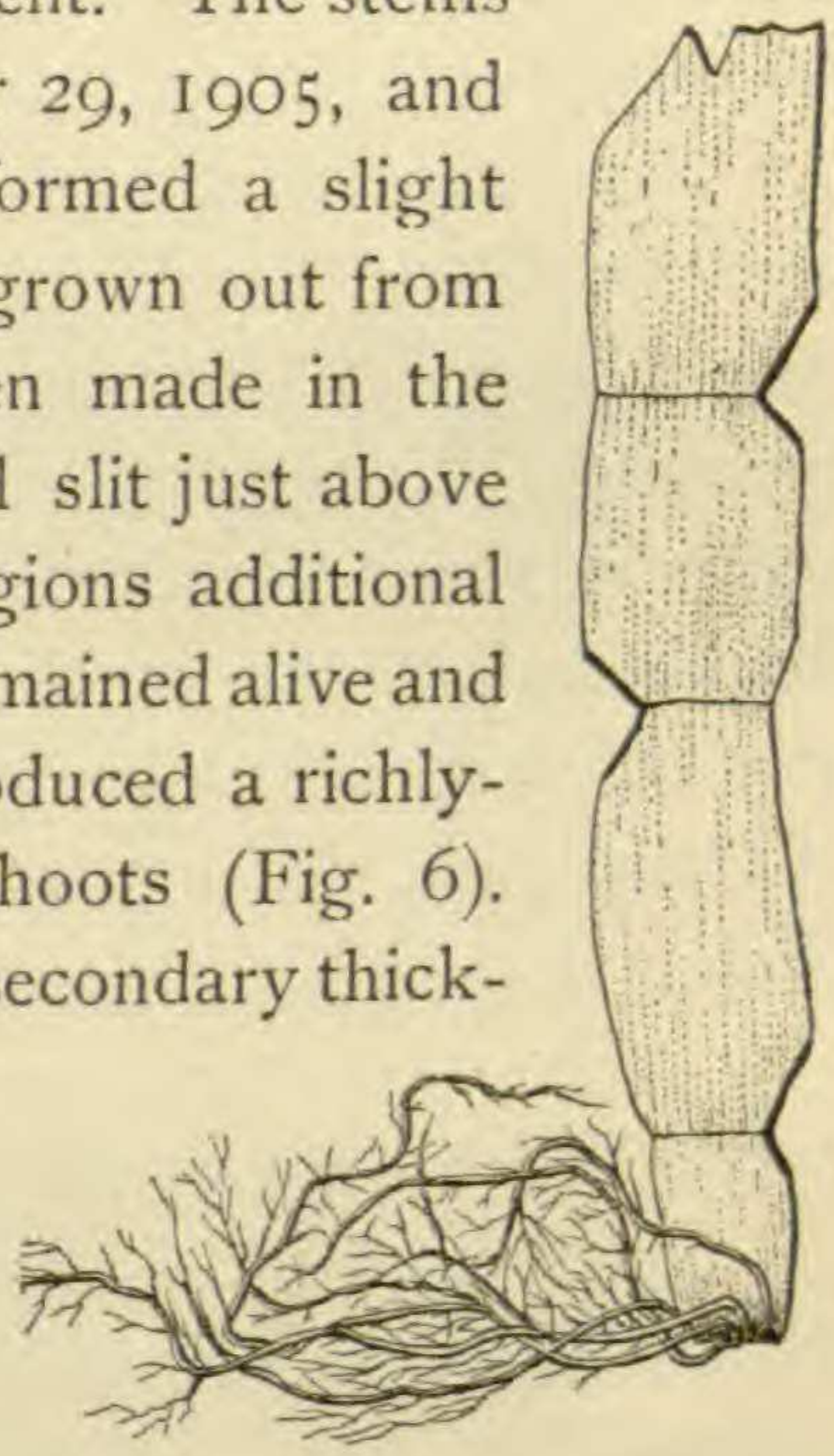

FIG. 6. Stem of *Muhlenbeckia platyclados* from which the buds were cut away. It regenerated only roots.

Experiment 15. — Eleven pieces similarly cut were planted in an inverted position. Seven of these failed entirely to root. The others produced a few roots, but never from the cut end of the internode. In these the roots came from the morphologically upper diagonal cut. The inverted pieces, probably because they had fewer roots also dried up after several months without having formed shoots.

Experiment 16. — To determine how far inward from the corner of the node the root and shoot-forming "impulse" extended, longitudinal slices of from 2 mm. to 4 mm. were cut from one edge of pieces from whose opposite side the scale-bearing region had been removed in the manner just described. These pieces were placed in a horizontal position in sand, with the straight edge down. In seven of the nine pieces thus placed, roots developed as in the erect pieces, from the cut end of the basal internode. The other two produced several small roots from the nodes on the straight side. One of the seven in which the cut end of the internode bore the roots, produced these as well from an oblique cut on the notched side of the stem, which was in the air, but not far above the ground. When eight pieces like those just mentioned were placed with the notched edge under the sand, in all cases, again, the basal internode developed roots, and in two pieces the upper oblique cut produced them as well.

These experiments prove, it seems to me, that the organ-forming "tendency," as it may be called for the present, in this plant does not extend very far in from the edge of the stem. When, by means of the deeper cuts, all the region which is ordinarily active in this formation is removed, the roots are formed, not at the nodes, but only at the basal cut. On the other hand, when less has been lost, or when the oblique cuts have not excised all of this region, the organs may be formed as well or exclusively at these points. Moreover the inability of the parts to form shoots after they have been removed indicates the very narrow limits within which this regeneration takes place.

Experiment 17. — On November 4, 1904, on one piece of *Muhlenbeckia* stem, the diagonal cuts were made along one edge, removing the buds, while the other edge was left uninjured. The whole side which had one bud present was placed in the sand. Roots developed from one of the lower uninjured nodes, and from the basal internode. The former were removed and did not grow out again. A shoot, however, developed from the axillary bud. On October 15, 1905, the piece was taken out and examined. It was found that the shoot had established a direct connection with these basal roots by means of a secondary growth of vascular tissue through the middle of the old stem (Fig. 7). On section, it was found that from interfascicular cambia new bundles had been formed between the old in this restricted portion. The number of bundles intercalated between the old ones was most often two, but in places, one or three ; and these bundles ran continuously from the roots into the new shoot. The thickening was much more pronounced on the upper side than on the lower.

The *Muhlenbeckia* stem, as it develops from the cutting or seedling, is round and leaf-bearing; but it very shortly widens out into the flat, thin, leafless organ, characteristic of the species.*

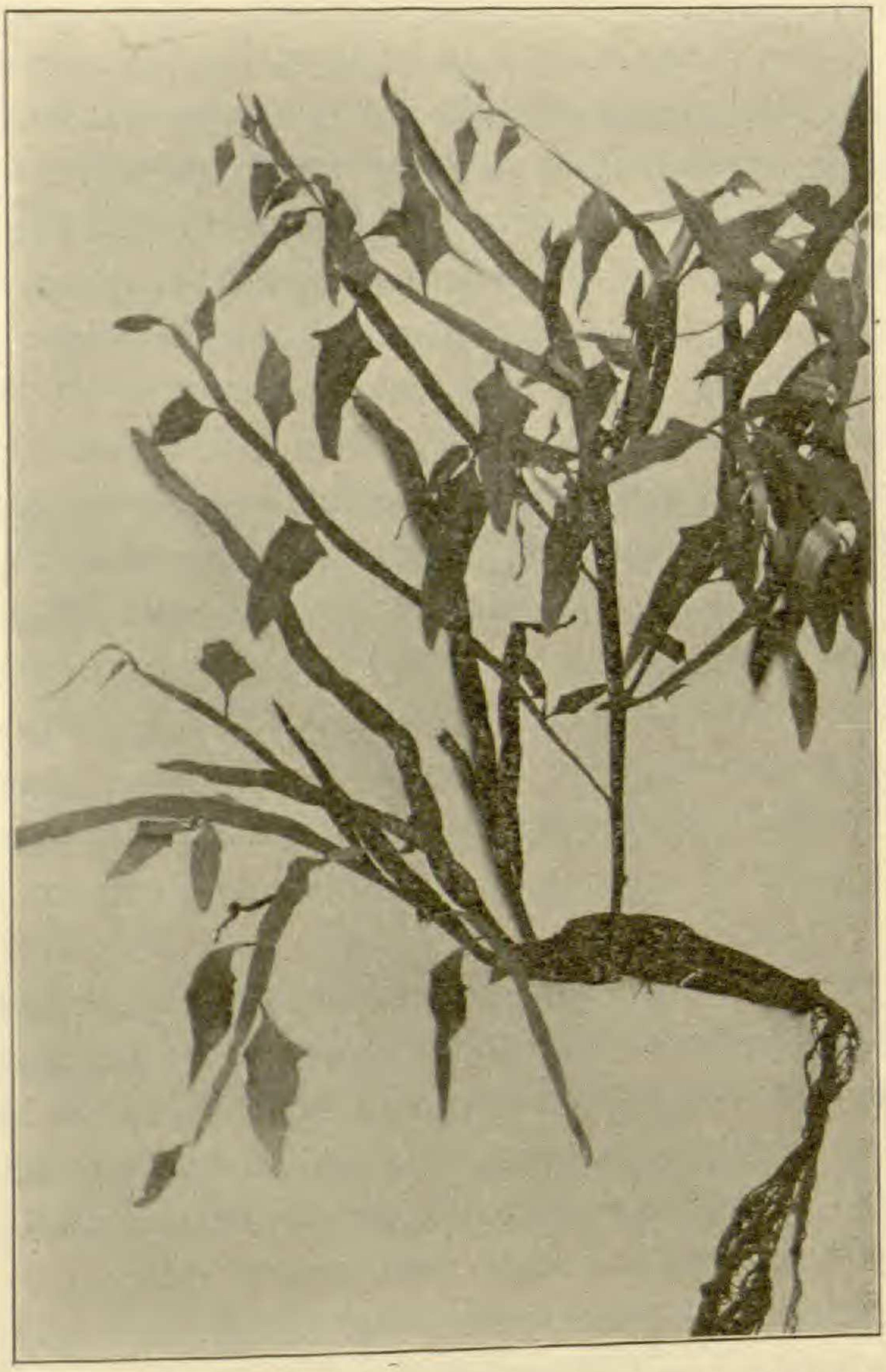

FIG. 7. Cutting of a stem of *Muhlenbeckia platyclados* in which the bud established a secondary vascular connection with the regenerated root.

A comparison between the round and flat portions of such a young stem shows that the number of bundles is approximately the same in the two, and that the difference in surface is accounted for by

* **Hildebrand, F.** Einige Beobachtungen an Keimlingen und Stecklingen. Bot. Zeit. 50: 2. 1892.

the increased amount of tissue between the bundles in the flat part. As the plant grows older, these flattened parts again become cylindrical. In this case the subsequent rounding-off is caused by the interpolation of new bundles between the old, so that eventually a continuous ring is formed, which thereafter widens from the cambium in the usual manner. The difference between this growth and that shown in the specimen of Fig. 7 is at once apparent. In the normal thickening, the whole stem changes its form, and the new bundles are formed at an equal rate in all parts. In the regenerated specimens, however, only about a third of the stem took part in the change, while the rest remained flat and unthickened as before. Even in the rounded part the new bundles had formed unevenly, so that every little elevation was visible on the reverse side from that shown in the figure. The unthickened edges would probably have been split off, in time, and growth would have continued regularly. Signs of such a splitting were just visible when the plant was removed for preservation. This behavior is also to be contrasted with that of the specimens from which all buds were cut (*Exp. 14*). Though the latter were growing for a period very nearly as long as the one just described, no secondary thickening took place. Evidently the presence of a growing point in some way regulates even a growth in diameter.

The stem of *Phyllocactus* resembles that of *Muhlenbeckia* in being flat and leafless. In other respects also, this plant recommends itself for the same series of experiments as the latter stem. Here only a very narrow triangle of tissue needs to be cut from the edge of a node in order to excise the normal bud-producing region. The bulk of the stem thus remains intact, and is able to continue its work of photosynthesis as before.

Experiment 18. — Portions of stems of *Phyllocactus* (sp.?) were subjected to incisions at the nodes similar to the ones described for *Muhlenbeckia*. When placed in an upright position they also produced the roots only from the lower cut surface. There is in these flat stems a rounder thicker central strand which is sometimes referred to as the "midrib." Most of the roots took their origin from the cut end of this strand, though an occasional root came from the flattened part. No shoots appeared. Laterally placed pieces with buds cut out have behaved in all respects exactly like the erect pieces.

In order to obtain information as to the behavior of the aerial shoots of the potato, before and after such excision of the axillary buds, experiments were set up in which at first only the basal bud and then progressively more buds were taken away until finally the cuts included the growing points of the stem.

Experiment 19.—The tops of such shoots, always exclusive of the lowest three internodes, were planted. The leaves were removed from the portion below the sand level. The parts rooted quickly but sparingly from the end of the internode; but when these roots were rubbed off, as frequently happened in taking up the cuttings for examination, no new ones formed. Notwithstanding this, the lowest axillary bud, which had been present at the beginning of the experiment as a small green rudiment in which the leaves were just visible, after five weeks became changed into a small tuber. This grew right up against the stem without any visible stolon.

Experiment 20. — The buds at all the nodes under the sand level were next removed. Roots were produced, as before, and at the first node above the earth a tuber was formed on a short stalk. It became green through exposure to the light, and the small leaves could be seen on it. When the leaf remained attached, a sessile tuber was formed in the axil.

Experiment 21. — In this series only the buds at a few of the apical nodes were allowed to remain. Again at these nodes, stalked tubers grew which were 15 cm. above the sand.

Experiment 22. — Finally all the buds, including the terminal buds, were excised in twenty-two pieces. Roots again formed, but in no case did a shoot regenerate. The pieces remained in the sand two and a half months but were not able to produce either a side branch or a tuber at any point.

The results are significant from several points of view. They show the ease with which a tendency present in a part may be shifted to another when its manifestation is rendered difficult at the usual place. Normally it is only a few of the lower nodes from which tubers are produced. Moreover, the experiments yield additional instances of tuber-formation in the light, which has been recently discussed by Vöchting,* Gager, † and others; ‡ and finally no better illustration could be cited of the difference in the reactions of parts possessing buds and parts deprived of them.

Regeneration in budless parts of potato-tubers was carefully worked out by Rechinger.§ He succeeded in inducing the formation of a shoot from cubes 4 cm. in diameter cut from the center of the tuber. He traced the origin of the scanty callus formed to the cambial strands which ramify through the stem, and showed that the shoot always arose in the neighborhood of such a callus. When he removed the "eyes" of the potato and cut the remainder into halves or quarters, shoots formed on the cut surfaces. Some experiments performed before the writer had learned of Rechinger's work may be worth mentioning, as they showed some slight differences from his results.

Experiment 23. — From a series of potatoes, the buds, together with approximately 1 cm. of underlying tissue were removed. After a period varying considerably on the different occasions on which the experiment was performed, buds appeared on one of the surfaces exposed by cutting out the original buds. The "reaction-time" in these cases varied from thirty-six days to five months, and one potato lived fourteen months without regenerating or decaying. This variation was independent of the season of the year, as was proved by the fact that great differences were observed in similar seasons of recurring years. Nor was it slowest in the fall and quickest in February, as might have been expected from the normal sprouting habits of uninjured potatoes. The differences were doubtless due to the fact that no one variety of potatoes could be secured for all the experiments, and that no definite information could be obtained as to the names of the varieties purchased.

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* **Vöchting, H.** Über die Keimung der Kartoffelknollen. Bot. Zeit. **60**: 86. 1902.

† **Gager, C. S.** Tuber-formation in Solanum tuberosum in daylight. Torreya **6**: 181. S 1906

‡ Knight (Phil. Trans. 1806) found that when the tubers were pinched off from the lower nodes, as they appeared on the plant, these were formed above ground, in the light. As far as I know, however, the behavior of cuttings in this respect has not before been recorded.

§ **Rechinger, C.** Verh. zool.-bot. Ges. Wien **43**: 315. 1893.

Experiment 24. — Parts with eyes removed in the manner described were cut into halves and quarters like Rechinger's and planted with the cut end down. These formed shoots, though not, as in Rechinger's results, at the large cuts, but in the region of the original bud. In no case was a shoot found on such a cut.

Experiment 25. — Pared potatoes as wholes and in parts were next tried, but although some of these resisted decay for four months, no shoot ever appeared.

Experiment 26. — The buds formed on the potatoes of experiments 23 and 24 were repeatedly removed, but new ones continued to form, sometimes in the same place, sometimes at a different node. If the parts were left undisturbed, shoots finally appeared at many of the nodes as in the uninjured tubers. No polarity could be observed in the appearance of the buds. They were formed with apparent indifference at any of the cut regions, without reference to apex, base, dorsal or ventral surface. No roots were formed in the specimens under observation. Modified stems of other types — thorns, tendrils, and rhizomes from which the buds were removed did not regenerate.

Internodes differ from the type of stem described above chiefly in their smaller size, but also in the fact that all the nodal tissues in addition to that directly concerned in the production of the bud has been lost. Apparently for this reason, they disintegrate with far greater rapidity than the larger pieces. Only in the case of certain of the begonias have records of shoot-formation in internodes been met with. The results to be indicated re-emphasize the rarity of this occurrence.

Experiment 27. — The internodes of a series of plants regenerated in a manner similar to *Heterocentron diversifolium* described by Vöchting,* in that they gave rise to roots at the base, but not to shoots. Internodes which yielded this result belonged to *Muhlenbeckia platyclados*, *Phyllocactus*, *Vitis quadrangularis*, *Hedera helix* and *Bryophyllum calycinum.* Internodes of a large number of other plants decayed without regeneration.

Experiment 28. — Internodes of *Muhlenbeckia platyclados* were placed in inverted positions in the sand. On one piece, in the first trial, roots appeared, but the suspicion arose that it had been

* Über Organbildung, etc., 72.

accidentally restored to its original position. In none of the twenty-seven pieces in which the apical end had been marked for identification did roots develop. A very slight callus was formed, but this soon went over into cork-formation. Normally, then, these inverted internodes do not root.

Experiment 29. — A similar result was obtained from two inverted internodes of *Vitis quadrangularis*. Lack of material prevented further work on this plant.

Monocotyledonous stem pieces without buds were generally found to give negative results even as regards root-formation. This may be due to the absence of a cambium to initiate callus-formation, but, on the other hand, it is well known that callus may originate from almost any parenchymatous tissue. The failure to root was probably due to an unfortunate choice of material for experimentation. One monocotyledon, however, gave an interesting result. The "pseudobulb" of the orchids is morphologically a short, thickened stem consisting of one or several internodes, in which growth ceases after a single season. In *Dendrobium Parishii* the pseudobulbs usually have four such internodes.

FIG. 8. Pseudobulb of *Dendrobium Parishii* which has formed roots and a shoot at the base.

Experiment 30. — One of these stems two years old was placed in sand on November 4, 1904. On February 18, 1905, the presence of roots of the aerial type at the base of the cutting was discovered, and the part was transferred to peat. By October 15, 1905, a new shoot had appeared, also at the base. The single leaf characteristic of the species was plainly visible (Fig. 8).

Because of the well-known difficulty in inducing conifers to root,* cuttings were made of seedlings of these plants and of stems of plants in their fourth year.

Experiment 31. — When the entire root was removed from seedlings of *Pinus Laricio*, a callus was formed after four weeks at the end of the hypocotyl. From this in seven out of the nine cases in which organs were formed, a single root, occu-

* **Sorauer, P.** Handbuch der Pflanzenkrankheiten, II Auflage 1 : 663. Also Populäre Pflanzenphysiologie für Gärtner, 169. 1891.

pying the whole of the cut surface, was regenerated (Fig. 9, *a*). Thus the seedling was "restored" to its original condition. Sections showed that most of the callus formed had gone directly over into the formation of the one root. A few rows of cells on the side,

FIG. 9. *a*. Seedling, and *b*, older stem cutting of *Pinus Laricio*. In both a single root regenerated from the callus of the cut surface.

however, which were not so used, showed that another root might have been formed therefrom if there had been space for its development. In the other two cases, two roots were formed from the beginning. Prantl * and Simon † mention instances in

* **Prantl, K.** Untersuchungen über die Regeneration des Vegetationspunktes an Angiospermenwurzeln. Arbeit. Bot. Inst. Wurzburg 550. 1874.

† **Simon, S.** Untersuchungen über die Regeneration der Wurzelspitze. Jahrb. wiss. Bot. 40: 116. 1904.

roots, where, after the removal of a piece from the tip of between 1 mm. and 2 mm., a single root may form from the cut instead of more, as is common. No case has been found by the writer in which a single organ is recorded as coming from the stem, when all of the root had been cut off. Here the sympodial character of the regenerated organ becomes obscured and the seedling externally resembles the uninjured one in all essentials.

Experiment 32. — The stems of each of the three-year-old plants of this pine were cut into four pieces and planted. All of these produced slight calluses, but in the two lower sets no roots formed, even when the callus was again wounded. One of the apical pieces, however, formed a root directly from the stem above the callus — a phenomenon that has been often observed in other instances; and in one of the second set, as in the seedling, a single root appeared from the middle of the callus which grew so as to occupy the entire cut surface, again restoring the plantlet to its original condition (Fig. 9, *b*). Thus there seems to be a tendency in this pine to form only one root from the callus produced as a result of injury. Not many instances of this restoration in adult tissue are known.

The great majority of plants with aerial stems (the beech is the best known exception) — are capable of being propagated from stem-cuttings. It is, therefore, instructive to reflect that this power, is, in at least a large percentage of cases, due entirely to the presence of shoot-buds on the part before the cutting is made. This is not universally true. The cut-stems of poplars, elms, and other trees are known to develop shoots from a callus on their apical cut ends; and Vöchting obtained shoots from the internodes of *Begonia discolor*.* But in most cases, when such buds are removed, the stems seem to be incapable of replacing them, and the cuttings fail to establish themselves as plants. The behavior of such parts serves to emphasize the principle that while unfavorable external conditions may prevent a normal regeneration or cause a change in its position, even the most auspicious culture conditions fail to induce such a formation when the internal impulse is lacking.

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* *L. c.* 78.

REGENERATION IN LEAVES

Leaves are organs which usually upon removal from the plant, without further incisions, are freed from connection with preformed rudiments. A few (*Bryophyllum crenatum* and *Leptaxis Menziesii*) have normally such primordia, which had to be detached before the leaves could be included in the limits of the present work. Besides a repetition of the experiments with normal leaves as wholes and in parts, the behavior of modified leaves of different types has been investigated; among the latter figured thorns, phyllodes and so-called "juvenile" leaves. In connection with the regeneration of the bean leaf, an anatomical study of callus and root-formation in this species was undertaken; and, finally, the power of *Leptaxis Menziesii* to replace its bud after its removal has been re-examined.

Of eighty-two species of green leaves used as cuttings only a very small proportion needs recording. The majority conform in their method of producing root and shoot — or, more often, the former only, to the descriptions given everywhere in botanical literature.* Vöchting,† in his discussion on the behavior of these organs, expressed the opinion that the leaf in which the power to regenerate a new plant is lacking, would, upon investigation prove to be the exception, rather than the rule. This has not been borne out by the following experiments. In all of the sixty-one species which gave any positive results, roots were formed, while only twelve kinds gave rise to shoots. All of the latter, with the exception of *Piper canescens* had been known before to have this property, so that out of 71 species in which the regenerative power had not been recorded, only one was discovered which was able to produce a new plant.

In 1816, Knight ‡ planted in earth the leaves of the potato, expecting them to root and to form tubers at the base. He was surprised that neither of these things happened, but that the end of the petiole swelled up, and was found to contain material like that in the tuber. (*Exp. 33.*) Consequently the ease and regularity

* For literature see **Vöchting** *l. c.* 92 *seq.*

† *L. c.* 97.

‡ **Knight, T.** On the action of detached leaves of plants. Phil. Trans. 289. 1816.

with which potato leaves so planted produced roots and, for a long time nothing else, caused the writer some astonishment (Fig. 10, *a*).

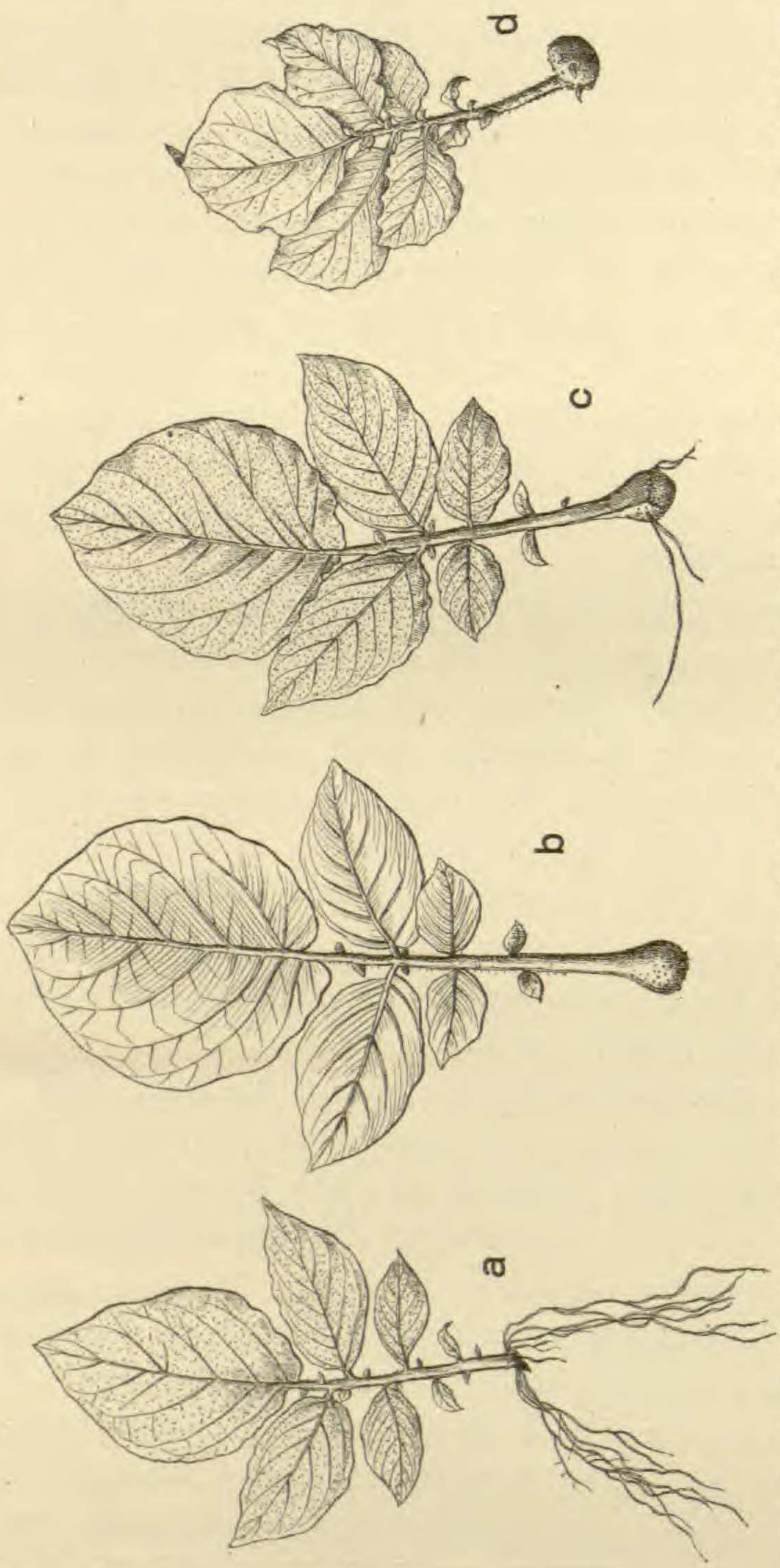

FIG. 10. Series of regenerating leaves of potato (*Solanum tuberosum*). *a*, showing regeneration of roots only; *b*, drawn from the under side showing swelling at the end of the petiole containing starch; *c*, swelling and roots present; *d*, with true tuber at the base.

At last a series was found in which the phenomenon described by Knight recurred (Fig. 10, *b*). Several leaves showed gradations between this type of regeneration and the preceding one in that they possessed both the tuberous swelling and roots (Fig. 10, *c*). And, as a climax one leaf has been discovered in which neither the roots nor the swelling appeared, but an actual tuber on which buds are visible (Fig. 10, *d*). All these leaves were taken from the same plant and subjected to apparently identical conditions.

Experiment 34.— When the petiole of a leaf is largely or entirely cut away, roots form additionally or solely from the under side of the lamina. This occurs, for example, in *Begonia Credneri*, *Peperomia argyrea*, *Iresine Herbstii*, etc. Roots may also be called forth by extra transverse cuts in the blade, and in *Euphorbia nivulia* by longitudinal cuts. Parts of leaves used as cuttings behaved just like those described by Vöchting and his predecessors; but in no case was an isolated petiole found to form shoots.

Experiment 35. — To determine whether leaves had any power of rooting from the apex, the tip was removed from blades of various species of *Begonia*, *Echeveria*, *Sedum* and *Pelargonium*, and from *Bryophyllum* leaves from which the buds had been cut out, and the leaves were planted with the apical end down. In order to prevent evaporation from the exposed end of the petiole, this was covered with paraffin. Some of the leaves remained turgid for five months, but none ever rooted in this position.

The abnormal increase in the size and thickness of regenerating leaves has been noticed by DeVries * and Lindemuth,† and has recently been worked out in careful detail by Mathuse.‡ The same phenomenon attracted the writer's attention and measurements were being taken when Lindemuth's paper appeared. His results and Mathuse's fail to confirm a statement made by DeVries that the life of a leaf when planted is not noticeably prolonged beyond its duration on the parent plant. Lindemuth's rooted leaves in some cases lasted five years and were then sacrificed for anatomical study. As further evidence that this statement is not universally true the following experiments with leaves which have a noticeably short lease of life on the plant may be mentioned.

* **DeVries, H.** Über abnormale Entstehung secundärer Gewebe. Jahrb. wiss. Bot. **22**: 35. 1891.

† **Lindemuth, H.** Über Grosserwerden isolierter ausgewachsener Blätter nach ihrer Bewurzelung. Ber. Deut. Bot. Ges. **22**: 171. 1904.

‡ **Mathuse, O.** Über abnormales sekundäres Wachstum von Laubblattern, etc. Bot. Centralb. Beiheft. **20**: 174. 1906.

Experiment 36. — *Euphorbia nivulia* is a xerophyte which is leafless except at the growing apex. In the plants that have been under observation in the greenhouse, moreover, these new stems bear leaves for only a few months in the year. When planted, however, the leaves lived for over fifteen months in the sand. At the end of that period, while still in a healthy growing condition, they were transferred to a pot of fertilized soil in an attempt to induce shoot-formation. Very shortly after the transfer the entire pot and its contents unaccountably disappeared.

Experiment 37. — *Muhlenbeckia platyclados* also has leaves which are found only in the "juvenile" condition and form part of the plant for only a very limited period (see Fig. 7). Yet upon being placed in the sand, they also rooted and maintained themselves for several months, though not so long as *Euphorbia nivulia.*

Küster * makes a statement that thick leaves form calluses very readily, as a rule, while thin leaves or those containing a great deal of water usually produce only a very weak callus. For this reason, he continues, cotyledons usually are richer in this respect than later leaves. This has not proved to be the case with the leaves of the kidney bean (*Phaseolus vulgaris*), lima bean (*Phaseolus lunatus*) or lupine (*Lupinus alba*). The primary and later leaves of the bean, which are very thin, form a larger callus and root more easily than any other leaves which have been tried. Their regeneration takes place with considerably more certainty and rapidity than that of the cotyledons, which very frequently decay without forming calluses or roots. The lupine cotyledons also form weaker calluses and regenerate more slowly than the later leaves.

The leaf of the bean forms a callus within a few days after being planted, and roots develop between the ninth and the thirteenth day. The question as to the point of origin of such roots has been much discussed. Stoll,† disputing a statement made by Cruger ‡ that roots could arise either in the callus or above it, maintained that the callus formed no vegetative points, but that

* **Küster, E.** Pathologische Pflanzenanatomie 168. 1903.

† **Stoll, R.** Über die Bildung des Kallus bei Stecklingen. Bot. Zeit. **32** : 736. 1874.

‡ **Cruger, H.** Einiges über die Gewebeänderungen bei der Fortpflanzung durch Stecklinge. Bot. Zeit. **18** : 369. 1860.

these always originated in the pericambial region above its level. Wiesner * and Hansen,† however, have confirmed Cruger's results and there is no doubt that organs may arise in both ways. The pulvinus of the primary leaf of the bean was cut off, so as to simplify the anatomical study, and the leaves were planted. Every twenty-four hours up to the ninth day on which, in some cases, roots became visible, specimens were removed and killed. Microtome sections showed that the callus-formation begins in the cortical region just outside the bundles on the second day. There may be several centers of such formation in the rind, which start independently but later unite. On the fourth day activity is noticeable in the pith cells, as well, of which there are a few rows surrounding the cavity of the hollow petiole. The callus resulting from the growth in these two regions eventually, about the seventh day, covers over the entire cut end of the petiole so that the hollow is no longer visible. Roots may grow out in both of the ways described above. Sometimes the callus remains sterile and the roots appear only from the petiole above. At other times they start in the part of the callus which covers the cavity, so that there can be no question here of a derivation from a cambial tissue at a higher level. The stages in the formation of callus and roots closely coincide with the processes as described by Stoll,‡ Tittman,§ Sorauer || and others, so that there seems no need of a further description here.

Experiment 38. — The callus was allowed to form fully, and was then cut off on the eighth day. Others were repeatedly removed after the same lapse of time, and it was found that a single leaf could form eight such calluses before it disintegrated.

Experiment 39. — Bean leaves which had formed calluses and roots were transferred to a rich loam, to a soil saturated with culture solution, and to a one per cent solution of grape sugar, to

* **Wiesner, J.** Die Elementarstruktur und das Wachstum der lebender Substanz 94. 1892.

† **Hansen, A.** Vergleichende Untersuchung über Adventivbildungen im Pflanzenreich. Abh. Senckenb. Naturf. Ges. **12** : 157 *seq.* 1881.

‡ **Stoll, R.** *l. c.* 752 and *pl. 12*.

§ **Tittmann, H.** Physiologische Untersuchungen uber Callusbildung an Stecklingen holziger Gewachse. Jahrb. wiss. Bot. **27** : 164. 1895.

|| **Sorauer, P.** Handbuch der Pflanzenkrankheiten. 2 Auflage **1** : 660 *seq.* and *pl. 13*.

see if a better food supply could induce the production of shoots; but these organs did not develop.

Of a large variety of phyllodia, leaf-thorns, leaf-tendrils and pitchers planted, only *Acacia pycnantha* yielded positive results.

Experiment 40. — Its phyllodia used as cuttings rooted with great apparent difficulty after seven months, and subsequently decayed without further regeneration.

A modified leaf of a different type is represented by the bulb-scale. The method of multiplying hyacinth-bulbs by cutting off the base and allowing the separate scales to regenerate, has long been practiced by the Dutch bulb growers.* *Lilium tigrinum* has been found by Beyerinck † to have the same faculty. Other bulbs tried — tulip, oxalis, daffodil — did not exhibit this power. The scales of the hyacinth do not themselves root, but the bud, shortly after it is formed, develops a root of its own.

Experiment 41. — Scales of the onion bulb, on the other hand, produced roots with little difficulty, but never gave rise to any buds. In one case an onion gave a very deceptive appearance of rooting from the apex of the scales, as well as from the base. A closer examination, however, showed that the roots had arisen from the base, as usual, but had pushed their way up between the inner epidermis and the mesophyll of the scales emerging at the top.

As an addition to the number of plants in which shoot buds can be induced to form while the leaf is still on the plant may be mentioned *Sedum tortuosum.* This leaf has no preformed rudiments, but can, when removed, form root and shoot in the normal manner at the base. A transverse cut had been accidentally made in the middle of a blade, and when the plant first attracted notice, it had formed a bud at the cut.

Experiment 42. — Similar wounds were made, experimentally, and in several cases the bud appeared (Fig. 11). In some, root rudiments were visible on the lower surface, but their development was suppressed by the dryness of the air. Other types of cuts —

*Fortune, R. A Visit to the Bulb Farms of Haarlem. Gard. Chron. 556. 1863.

† Beyerinck, M. W. Over het ontstaan van Knoppen en Wortels uit bladen. (Reviewed in Bot. Centralb. 14: 112. 1883.)

longitudinal, tangential, and transverse cuts from the under surface, have failed to call forth any regeneration.*

Wakker † divides leaves which regenerate into those in which buds are normally present in the life of the leaf, and those in which they are absent. He says that if the buds are removed

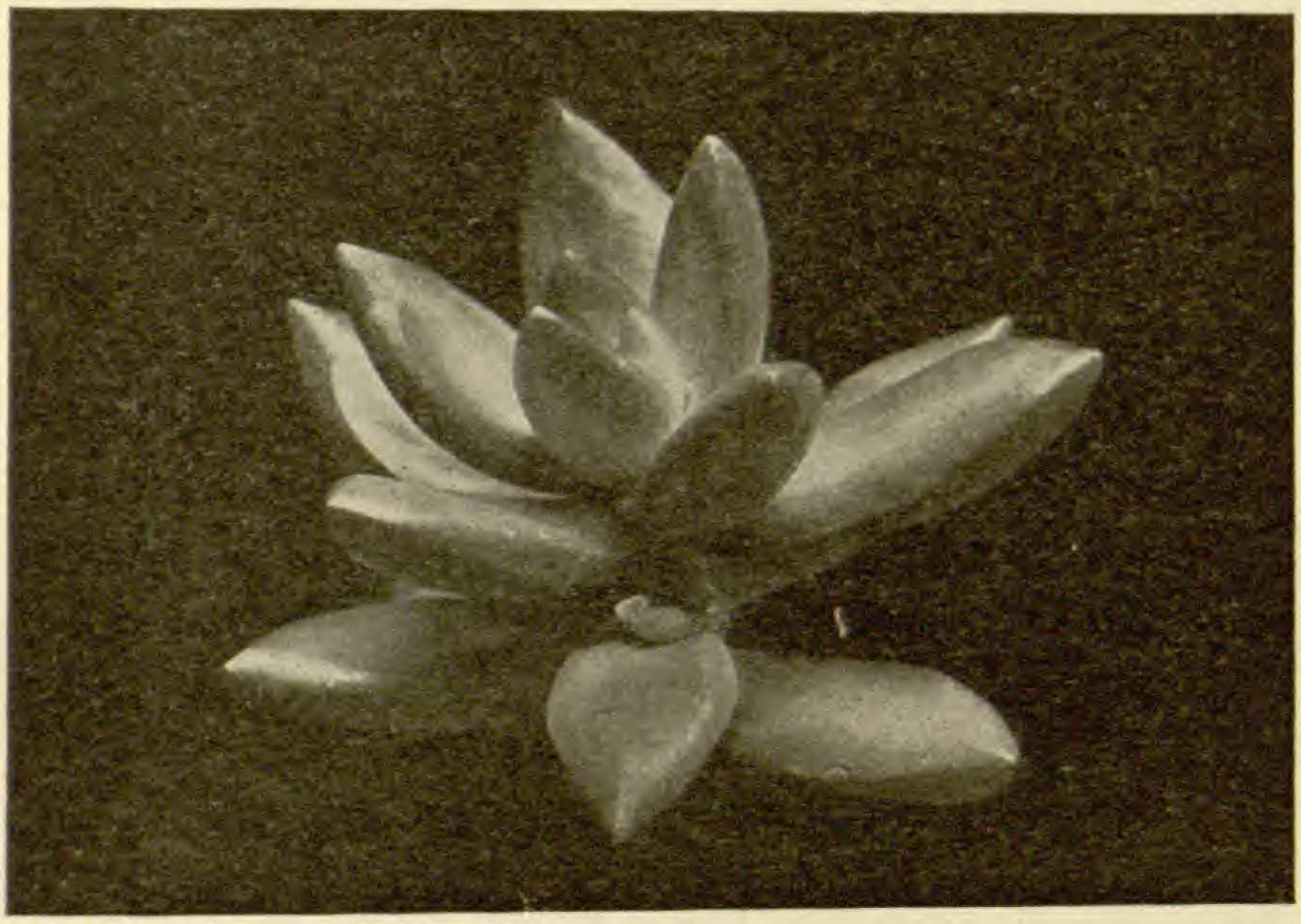

FIG. 11. Shoot of *Sedum tortuosum* showing a bud at a transverse cut in a leaf.

from the leaves of the former type — of which *Bryophyllum* is the best known example, they cannot be replaced. The results of an operation of this sort on *Leptaxis* (*Tolmiea*) *Menziesii* show that this is not universally true.

Experiment 43. — The buds which are situated at the point of junction of the blade and petiole of these leaves were removed with a very sharp small scalpel from twelve specimens, so that no visible trace of these remained. Then the leaves were placed in the sand. They rooted plentifully both from the preformed rudiments at the back of the petiole, near its insertion, and from new

* An interesting fact (which is well known in other instances) was illustrated by this plant. Though it had been readily propagated from stem and leaf cuttings, and had been placed provisionally under the name of *Sedum aureum*, the plant had never been known to flower. In the regeneration experiments, practically every leaf was wounded in one way or another; as a result a flower stalk appeared, and the probable identity of the species was discovered.

† **Wakker, J. H.** Onderzoekingen over adventieve Knoppen. (Reviewed in Bot. Centralb. **32**: 238. 1887.)

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† **Beyerinck, M. W.** Over het ontstaan van Knoppen en Wortels uit bladen. (Reviewed in Bot. Centralb. 14: 112. 1883.)

which did not appear again in this or in either of the other parts. In a similiar trial with peduncles of *Bryophyllum crenatum*, only the lowest of the three parts rooted, and no shoot was produced.

Experiment 45. — *Ruellia rosea* has an inflorescence of a different type. The peduncle is green and slightly flattened, without leaves. In the seventeen months in which the parts remained alive, they regenerated an ample root-system, but nothing further.

REGENERATION IN FRUITS

Few instances of regeneration in fruits have been recorded. In some of these the fruits are of the type of *Opuntia* where the stem is incorporated in the fruit.* Roots have also been reported on the capsule of *Lilium speciosum*.† Two additional green fruits proved, in these experiments, to have the faculty of producing roots.

Experiment 46. — Half-grown pods of yellow bush bean and lima beans were placed with their pedicels in sand. From the end of this stalk a callus was formed, and roots grew through this in three weeks (Fig. 12). In only one or two cases did the regenerated organ enable the pod to mature its seeds. Ordinarily the ripening was effectually prevented by the processes of decay which set in shortly after the roots were formed and eventually killed the part. Roots sometimes started from the stem above the callus, instead of growing through it, so that it is to be presumed that both sorts of origin are possible here as in the leaf. It must be admitted that this kind of regeneration is fundamentally like that of an inflorescence. That there is a difference, however, was shown by the following comparison.

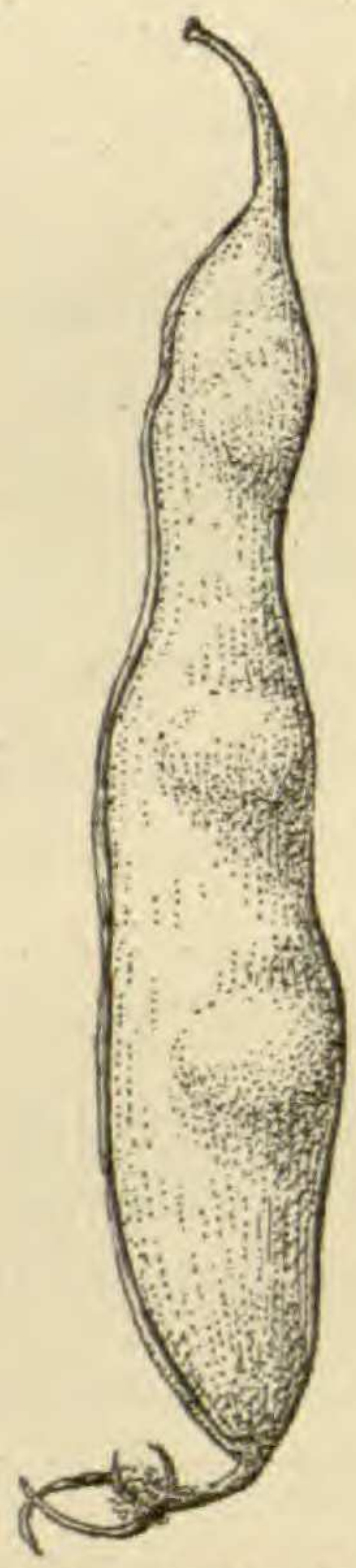

FIG. 12. Pod of *Phaseolus*, which has formed a callus and roots from the end of the pedicel.

* **Vöchting.** *l. c.* 110; also **Hildebrand, F.** Über Bildung von Laubsprossen aus Bluthensprossen bei Opuntia. Ber. Deut. Bot. Ges. **6** : 109. 1888.

† **Carrière, E. A.** Un Fruit qui s'enracine. Rev. Hort. **49** : 207. 1877.

Experiment 47. — Bean flowers on their pedicels were placed in the frame beside the half-mature pods. The former always died without regeneration, while the pods rooted easily. The difference is doubtless to be ascribed to the smaller amount of material available for regeneration in the flower than in the fruit.

Experiment 48. — When the pedicel is cut off, or pulled off, and the pod alone is planted, it succeeds in forming a small callus, but no roots. There is, however, little doubt that such a part has the power of producing roots and that better manipulation will make this manifest.

The foregoing experiments have demonstrated that every part of the plant, even when preformed rudiments are absent, has some power of regeneration, though in a majority of the cases this is not complete enough to establish a new plant. One fact, furthermore, is brought into undoubted prominence as a result of the experiments, with so few exceptions that these are practically negligible. This is, that the disposition for root-formation is much more widespread throughout the plant and more easily energized than the power to produce shoots. The proportion of parts which formed only roots to those in which both sets of organs were regenerated was as 74 : 20. It has been seen, too, that where both kinds are produced, the roots usually precede the shoots by a period of time varying from three weeks to seven months. Only three instances were found in which the cuttings regenerated shoots alone — the potato-tuber, the central tissue of the horseradish-root and the hyacinth bulb-scale. In a certain sense, however, even these parts might be said to share in the production of roots, for the regenerated shoots almost immediately after they themselves become visible, form root primordia. On the very youngest buds of the potato these are present, and become developed before the shoot is further differentiated. The converse has never proved true in these investigations.* In the case of the *Pelargonium* leaf-cutting (*Exp.* 13) a shoot did spring from the root. However, this shoot was not the first one formed; it appeared only after a lapse of seven months and as the result of a second injury. An attempt at an

* Though Vöchting and Beyerinck state that it is often the case in woody roots of trees.

explanation for this phenomenon involves a consideration of the various theories of regeneration, and may, therefore, be deferred until this subject is discussed.

The experiments throw some light also on the dependence of organogeny upon the presence of a cambium, and upon callus formation. That the structures regenerated do not necessarily derive their origin from a cambium, as Stoll,* Frank,† and their followers assert, has been demonstrated by the results with isolated root-regions from which the cambium has been carefully cut away, and from the anatomical study of the bean petiole. It will be recalled that the central part of the parsnip, consisting only of wood and pith, was able to produce a callus and roots. Root-meristems, too, were seen to arise in the callus covering the hollow of the bean petiole. And, as to shoots, isolated rind-tissues of the parsnip were found to produce a callus from which buds arose (Fig. 4). The absence of any cambium or transverse bundles in this region was confirmed by a microscopical examination at the time the buds appeared. Coulter and Chrysler found a similar development of shoots from isolated cortical regions of *Zamia*.‡ There can be no doubt, therefore, that both roots and shoots may arise from callus that has no direct connection with cambium or bundles. That both sorts of organs can also spring from a cambium without the intermediation of a callus seems equally clear. Rechinger § describes such a derivation of shoots in the horseradish, and of course the origin of roots in such a manner is a common occurrence. The cells of both these tissues agree in being undifferentiated, rich in protoplasm, and capable of rapid division — three characteristics which seem to supply the structural basis for the appearance of primordia of root and shoot.||

Before closing this section of the work, it may be worth while to record the result of an experiment performed on the alga *Penicillus capitatus* in the summer of 1904 at the Flatts Harbor in

* *L. c.*, 761.

† **Frank, A. B.** Die Krankheiten der Pflanzen 1 : 70.

‡ **Coulter, J. M.**, and **Chrysler, M. A.** Regeneration in Zamia. Bot. Gaz. 38 : 452. 1904.

§ **Rechinger, C.** *L. c.*, 323.

|| A few instances are known, as for example in the epidermis of *Begonia* leaves, where organs arise from mature cells without even the intermediation of a callus.

Bermuda. The "shaving brush," as the alga is popularly called, is a siphonaceous form in which stalk and head are made up of branching filaments without cross walls. The stalk is encrusted with lime, but the filaments of the head are soft and flexible.

Experiment 49. — The "heads" of a number of these algae which were growing in the sand on the bottom of the harbor were cut off, and the algae were marked for identification. After 38 days it was found that a new head of loose filaments had begun to form (Fig. 13). On microscopic examination it appeared that the cut ends had closed over and the filaments had gone on branching in the normal manner. This alga, then, adds another to the list of the Siphoneae which have a considerable power of regeneration.

FIG. 13. *Penicillus capitatus.* After the "head" had been cut off a new one was formed.

The position and character of the new organs which appear on a part in regeneration have been accounted for in a great many different ways. Some of these influences are to be found in the external conditions to which the part is subjected, others in qualities and tendencies inherent in the part before injury. Though it has generally been assumed that an available food supply, either as stored nutriment or as the product of the photosynthetic activity of the cutting, is a *sine qua non* of regeneration, few attempts have been made to confirm this fact by experiment. The necessity of food has, indeed, been recently denied by McCallum.* He found that pieces only 8 mm. long of the stems of bean seedlings from which the cotyledons had been removed, could, even when kept in the dark, develop the buds present in the axils of cotyledons; and Morgan, calling attention to the similar phenomena described in regard to animals, has declared that the parts may regenerate even under conditions of starvation.

The following experiments performed on various parts have led to a different conclusion.

Experiment 50. — A plant of *Begonia Rex* was kept in the dark for two days. Four leaves from this plant were then cut in-

* **McCallum, W. B.** Regeneration of Plants. Bot. Gaz. 40: 105. 1905.

to pieces of which an equal number were left in the light and in the dark for 17 days. At the same time portions of the leaves were preserved for microscopical examination. Both sets of specimens regenerated roots and shoots in that time, those in the light being noticeably larger. This result would fall in line with the one described by McCallum and might warrant the deduction that the regeneration is independent of food. The error of the conclusion, however, lies in the fact that there was still a considerable amount of starch in the leaf at the time of cutting. Sections made of the parts which had been preserved showed starch grains scattered throughout the leaf, but collected sometimes in large numbers in the neighborhood of the veins. This material, therefore, doubtless served as the basis for regeneration.

Experiment 51. — Leaves of a plant of *Begonia Rex* which was darkened for four and one half days were next treated in the same manner. Sections showed still a few isolated starch grains, so that even after this lengthy exclusion from the light, not all the food previously made had been consumed. However, in this case the difference between the two sets of parts was marked. All those subsequently exposed to the light formed roots and shoots normally, while the ones in the dark decayed without regeneration.

Experiment 52. — The same result was obtained with leaves of a bean plant that had been darkened only 48 hours. Without light the leaves did not form even a callus; when set in the light, calluses and roots formed, but more slowly than normally. These results seem to indicate, therefore, that regeneration does not usually take place in the piece during starvation. Consequently the question naturally arises as to whether food actually was lacking in the stem-parts used by McCallum.

To confirm the result as to the effect of the absence of light, parts were also placed in an atmosphere devoid of carbon-dioxide.

Experiment 53. — The materials used were leaf-portions of *Begonia Rex*, of two species of *Peperomia* and of *Pelargonium* which had all been darkened four days before the beginning of the experiment. These were placed underneath a bell-jar from which the carbon-dioxide was removed in the usual manner. None of the parts regenerated. The experiment was not so conclusive as the preceding ones, however, inasmuch as the apparatus had not been set up so as to allow a renewal of the oxygen. Still as a large bell-jar had been used to cover the cuttings which themselves were small, it is probable that the amount of oxygen would have sufficed for regeneration had other conditions permitted.

Further evidence of the dependence of regeneration upon either a reserve food-supply or the ability of the part to make food was afforded by white shoots of various plants.

Experiment 54. — In the variegated *Commelina* (sp?) among the shoots with green leaves and with green and white striped leaves, occasional shoots appear on which all the leaves are pure white. Such white shoots were used as cuttings and compared with shoots having striped or green leaves. Whereas the latter rooted within a week or ten days, the white pieces in repeated experiments unanimously failed to regenerate. The results obtained with *Commelina* were reinforced by the behavior of white shoots of *Oplismenus Burmannii* (the *Panicum variegatum* of the gardeners), of *Pelargonium* (Madame Solleroi), and of some white orange seedlings which appeared as sports in the greenhouse. In none of these species was the white shoot able to form roots. If, however, the leaves on the shoot contained even a very narrow green area (*Commelina*, *Pelargonium*, *Oplismenus*) the organs were produced.

It may be argued here that such parts are in a pathological condition, and that the failure to root was due to other causes than the absence of a food-supply. White variegation is believed to be caused by an oxidase,* which when present in quantity prevents the formation of chlorophyll in the chloroplast. Except for this absence of coloring matter, however, the cells of the white shoots showed no microscopical difference from the normal ones. They were as rich in evenly distributed cytoplasm, and their nuclei were apparently similar. Moreover, the fact that the possession of a single green stripe only 1.5 mm. wide enabled the shoot to regenerate seems to point forcibly to the conclusion that it is primarily the lack of the food manufactured by the chlorophyll which prevented the shoot from rooting. When this green stripe was darkened, as was done crudely by coating it with India ink, the regeneration, while not prevented, was delayed for from 4 days to a week beyond the average time.

* Woods, A. F. The Destruction of Chlorophyll by oxidizing Enzymes. Centralb. Bakt. Parasitenk. und Infectionskrankheiten 5 : 745. 1899.

Experiment 55. — Attempts to supply the lack of this nourishment by growing the pieces in various dilute sugar and peptone solutions repeatedly failed. In only one case did a shoot of *Commelina* grown in a one per cent sugar solution, form a root 4 mm. long. In all the other trials, though attempts were made to keep down the number of bacteria by frequent change of the solution and by the addition of small doses of copper sulphate, the parts decayed without giving positive results. It does not seem at all unlikely that with better culture methods this regeneration may be more often induced.

The foregoing experiments indicate, therefore, that normally the leaf or shoot has at the time of cutting sufficient reserve food to initiate the first stages of regeneration. When, however, this food is absent and its formation is prevented either by external conditions or by the parasitic habit of the part, regeneration is inhibited. Given the necessary food supply, the question next arises as to what other conditions are responsible for the appearance of the organs in regeneration. Vöchting in 1878 gave the answer with regard to external influences which all subsequent experiment has failed appreciably to alter. In an exhaustive series of investigations on the effect of light, gravity, water, pressure, and contact, Vöchting came to the conclusion that while external conditions may have a modifying or an arresting effect on the place of the appearance of the organs, these could not be considered as primarily the causes of regeneration.* As was before mentioned he ascribed the practically uniform position of the structures formed on root and shoot-cuttings as due to an inherent force in the plant which he termed "polarity." The fact that leaves fail to exhibit this polarity, but form both kinds of organs from the base, he explained as due to the limited growth of the latter organs as contrasted with the unlimited growth of root and shoot.

His conclusions have not found universal acceptance. Instances have been recorded where — as for example in *Bryopsis* † — the root-pole could, by the action of light, be changed into a shoot-pole and the reverse. In the higher plants, too, numerous cases

* *E. g.*, *l. c.* 82.

† **Noll, F.** Über die Umkehrungsversuche mit Bryopsis. Ber. Deut. Bot. Ges. 18 : 444. 1900.

of so-called "reversal of polarity" have been found. When, for example, the production of a callus at the apical end of a poplar stem is prevented by growth in dry air, or by a cap of sealing-wax, the basal callus produces the shoots ; and instances of reversal in root-cuttings of *Taraxacum* have been referred to already. The experiments recorded above showed organs in unusual positions in the case of the shoots on the parsnip and horseradish and the roots on thin slices of sweet-potato. Yet, as Tittmann first emphasized, only one pole is ever affected in these results, while the other produces its normal type of organ or nothing.* And, further, though by unusual conditions the position of the regenerated structure has been modified, the piece cannot be said to have altered its polarity ; for as soon as it is restored to favorable surroundings, it will produce organs in the normal way. That this "normal way" must have reference to any peculiarities which the uninjured plant manifests has been made clear by some of Goebel's † later work.

Klebs,‡ while admitting the polar tendency in cuttings, as a result of a series of experiments on both uninjured and cut willow twigs, came to the conclusion that this disposition could not be the determining factor in evoking the appearance of the roots. By local application of water he succeeded in inducing the apical end of twigs still on the plant to root; and in cuttings, by varying the temperature at the two ends or by inversion whereby the basal end extended into dry air he found that the roots developed only at the apex. He accordingly states that the real cause of the appearance of the roots lies in the fact that something is supplied which was previously present in insufficient quantity for development. In the case of roots, at least, this necessary factor is a local abundance of water. Roots, he says, normally appear at the basal end of cuttings because here there is a contact with moist earth and an absence of light. He criticises Vöchting's inversion-experiments on the score that in many species of willow the rind is too thick to be easily permeable to water ; but if these twigs are partially denuded of bark so as to allow water to enter, an equal number of roots appear at apical or basal end independently of polarity. He con-

* **Tittmann, H.** *l. c.* 167 *seq.*

† **Goebel, K.** Allgemeine Regenerationsprobleme. Flora 95 : 405. 1905

‡ **Klebs, G.** Willkürliche Entwickelungsänderungen bei Pflanzen, 96–124. 1903.

cludes that it is "in hohem Grade warscheinlich das jede Polarität umkehrbar ist."

Later experiment has not, on the whole, confirmed Klebs's position. McCallum * showed that roots could be produced even while the part was slowly drying ; no amount of water could bring about the formation of roots on the apical end of the *Muhlenbeckia* internode ; and as a final answer to this contention Vöchting † has written a recent paper, in which, working with the same plants and using similar methods to those of Klebs, he proved the latter's results and conclusion faulty. He found that when the twigs of this willow were cut into three pieces, all either erect or inverted, the basal piece always produced most roots ; that less roots were produced in water than in moist sand ; and that freeing twigs from cork was rather a hindrance than an aid to root-production. As a result he re-emphasizes his old position that in the higher plants, at least, polarity is a very stable, probably hereditary characteristic which it is a difficult matter to reverse. Just what the conditions were which caused the unusual position of the organs in the roots referred to above, has not yet been determined.

Goebel in interpreting this polar tendency at first adopted the Duhamel-Sachs "formative stuffs" hypothesis.‡ This theory held that there are in the plant definite root-forming and shoot-forming substances which usually, owing to the action of gravity, flow in different directions. The lighter shoot-forming stuffs flow upward and their aggregation at a cut causes the appearance of a shoot there ; whereas the heavier root-forming substances flowing downward account for the growth of roots on such lower ends. However the possibility of a flow of both stuffs in both directions independent of the action of gravity was finally admitted by Sachs to explain the presence of both kinds of organs in tubers and seeds. In Goebel's § later articles, he no longer insists upon different formative stuffs, but believes the phenomena capable of

* **McCallum, W. B.** *l. c.* 119.

† **Vöchting, H.** Über Regeneration und Polarität bei hohern Pflanzen. Bot. Zeit. 64 : 101–131. 1906.

‡ **Sachs, J.** Stoff und Form der Pflanzenorgane Gesamelte Abh. über Pflanzenphysiologie, 2 : 1159–1200.

§ See Regeneration im Pflanzenreiche. Biol. Centralbl. 95 : 385 *seq.* 1905 ; also Weitere Studien über Regeneration. Flora 92 : 132 *seq.* Ja 1903.

interpretation without this assumption. The vegetative points of root and shoot normally appropriate the food-material (not further specialized). When the connection with these growing points is interrupted either by mechanically hindering their development or by cutting the conducting system, the material flows to other previously dormant vegetative points and rouses these into activity. The new structures then appear at a nearby meristem if such exists. If a preformed growing point is absent, the heaping up of the food at the base of the interrupted conducting tissue, be it cut vein or the end of the petiole, causes the organs to grow here.

The other factors which have by different writers been considered causal in calling forth regeneration may be briefly enumerated. Noll * regards the form disturbance as the energizing influence; Küster,† the removal of the hindrances to growth; Wiesner,‡ the impulse from the dead cells at the wound to the living cells beyond. In a series of ingenious experiments conducted mainly on the cotyledonary buds of the bean, McCallum § has certainly succeeded in proving that no one of these factors is alone responsible in calling forth the development of these buds, which he regards as regeneration. His work, however, seems open to criticism on the score that he nowhere considers the possibility of two or more of these conditions working in concert to bring about these results; and for that reason his decisions that the "wound-stimulus is not in itself any part of the cause" and that the growth is not brought about "through any disturbance created in the nutritive or water relations" do not carry conviction. To his own conclusion, moreover, that the regeneration is due to the removal of "some influence which an organ . . . is able to exert over other parts and so prevent their growth," the same comment would apply as to the explanations which he criticises. Even when this condition is present, the formation of one or the other kind of organ may fail to take place; and on the other hand, the regeneration may occur without the separation

* **Noll, F.** Über die Körperform als Ursache von formativen und Orientirungsreisen. Sep. Abd. aus den Sitzungsb. Niederhein. Ges. 1900. 1–6.

† **Küster, E.** Pathologische Pflanzenanatomie 276.

‡ **Wiesner, J.** Die Elementarstruktur und das Wachstum der lebender Substanz 102.

§ **McCallum, W. B.** *l. c.* 243, 262.

from the influence of the parts above and below, which he considers necessary. When cuttings are made of stems from which the buds have been excised, the parts are certainly removed from the influence of both growing roots and shoots; yet, except in the potato-tuber, only roots are capable of being replaced. The tuber moreover, though separated from the roots of the plant of which it formed a part, itself does not acquire roots. That this disability is not due solely to correlations as one might think, is proved by the fact that even when the buds (which usually root immediately upon their development) are repeatedly cut away, the tuber is still unable to supply the loss of roots. Accordingly, isolation from either type of organ is not alone sufficient to call forth its production. Furthermore the bud can be induced to appear on the leaf of *Sedum tortuosum* and other plants without such detachment. Here by simply making a narrow transverse cut through the midrib, shoots and sometimes root-rudiments form on the leaves while they still retain their connection with both root and shoot of the growing plant. The same fact proved true of the roots formed both in his own experiments and in those of Klebs.*

As has been shown above, the replacement of roots either as direct restorations or as outgrowths from the cambium of the main or secondary roots ("Ersatzbildungen") is a more generalized faculty throughout the plant than the acquisition of shoots when rudiments are lacking; also when both kinds of organs grow anew the roots have almost always been seen to precede the shoots by a period of time that may be considerable. The explanations of regeneration above mentioned do not account for either of these phenomena. It is undoubtedly true that the aggregation of food at the end of an interrupted conducting system is, as Goebel maintains, one of the factors favoring regeneration. Yet again in the case of the stems with excised buds and in many leaves, though food unquestionably gathers at the cuts, this is not effective in bringing about the development of a shoot meristem. Moreover, in the cases of the inflorescences and fruits which regenerate, whatever current of food material occurs must be upward toward the developing flowers and seeds. Nevertheless, in all the instances

* *L. c.* 103.

in which the regeneration of these parts has been recorded, one or both structures are formed only at the base. This has always seemed to indicate a serious flaw in Goebel's hypothesis.

The teleological explanation of the early and general appearance of roots on nearly all kinds of plant-cuttings is a tempting one. It might seem as if roots formed easily because they are immediately necessary to the life of the part. But though the advantage cannot be denied, it cannot be considered as an explanation of their appearance. In nature, the opportunities for regeneration do not occur in a sufficient number of individuals to allow the faculty to become established by natural selection; and if only roots are formed, as so often happens, even the advantage is only transitory.

Other interpretations which readily suggest themselves also fail to hold on closer scrutiny. It might be thought that the simpler structure of the roots as compared with the shoots would account for the facility with which they are produced; but when a part has had at its disposal during many months building-material enough to construct an elaborately branched root system out of all proportion to the needs of the cutting, the question may well be asked why some of it was not at once expended in the generation of a shoot, which would thus have reproduced the plant. Nor can external conditions here be considered causal, since it has been shown that these may be varied radically with only arresting or slightly modifying differences in the result.

The only recourse, therefore, seems to be to go back to the old formative stuffs idea in perhaps a somewhat altered form. If a part fails to produce a certain organ when food is plentiful, and even massed near a cut, when all relations between the piece and other growing regions are severed, when all hindrances to growth are removed, and when external conditions are at an optimum for such formation, it would seem to indicate that what is lacking is a very definite substance which it is not always in the power of the cutting to supply. In what guise is this substance to be regarded? To assume with Sachs that the sap contains two different materials, one heavy and root-forming which moves downward, and one lighter and shoot-forming which moves upward, owing to the action of gravity, blocks, as Vöchting * has justly intimated, the way to fur-

* Bot. Zeit. 64: 101 *seq.* 1906.

ther investigations on account of the impossibility of detecting such materials, or explaining their flow.

The following theory is offered as a suggestion only, and does not pretend to account for all the phenomena in the field ; but as it is to a certain extent approachable by experiment, it may be worth while to present it at this point. Inasmuch as the polar tendency in a plant may be regarded as one of its hereditary qualities, the power to form roots or shoots at any point might be ascribed to the presence at such a place of particular enzymes, which are responsible for the formation of the organ in question. While the fertilized egg-cell would contain both of these enzymes, theoretically restricted to the opposite ends of the cell, this is not the case with the majority of cells resulting from its division. As growth of the plant continues, the shoot-forming enzyme becomes localized at the nodes where buds are being formed, at the growing points of the stem, and (in the case of roots which normally produce buds) in the neighborhood of the emerging secondary roots. The root-forming enzyme, on the other hand, apparently remains a permanent constituent of nearly all cambial cells throughout the plant. If we can remove the product of the activity of the enzyme but leave it behind, the regeneration of the organ becomes possible when food and growing cells are present. This would account for the fact that a vegetative point, when split longitudinally or when cut transversely very near the end, is able to replace directly what is lost ; but when the cut, extending a little farther back, presumably removes the enzyme as well, no restoration takes place. Instead a lateral organ produced from some other place where a sufficient quantity of the enzyme is still retained assumes the role of the axial organ which has been lost ; or, in the case where shoot-buds are excised from a stem, as practically all this sort of enzyme has been removed at the same time, no new shoots form.

The root-forming enzyme which is, according to supposition, present in nearly all cambial cells is normally prevented from acting by the fact that the growth is hindered by the surrounding cells and perhaps also by the absence of a static food material upon which to act. When a cut is made, however, the stimulus of the wound rouses the enzyme into activity, the food aggregating here supplies the material, and the organ, no longer hindered by encompassing tissue, makes its appearance. While, as was suggested above, the shoot-forming enzyme is usually restricted in its distribution, it can also sometimes be formed anew when cells which have by differentiation lost this substance, upon injury, resume more or less of their embryonic characteristics, in the formation of a callus.

Our entire knowledge of enzymes and their mode of action is almost in its infancy, but some of the more recent discoveries as to their history and condition of activity may apply here. For example, it is known that an enzyme may be present in a cell not as such but in the form of a pro-enzyme or zymogen which changes into the enzyme upon certain kinds of stimulation. Such might well be taken to be the case in the root-forming enzyme, where the stimulating factor could be the exposure of the cells containing the zymogen to moist air, or this condition in combination with others, such as a change in the pressure relations and the chemical effect of aggregated food. Again, the presence of a substance called a *kinase* is known to enhance the action of an enzyme, and it may be that such a substance, in addition to the enzyme is present at the nodes and vegetative points. Finally, as a third possibility, cases are known where, in company with an enzyme, a compound of an opposing nature, termed an "anti-enzyme" exists, and according as one or the other gains the ascendant the particular effect of the enzyme is manifest or obscured. Some such explanation might account for the individual variations in the regeneration of the same part under like conditions, as for example, the differences observed in the behavior of the potato leaf. However, while the possibility of the existence of such kinds of substances should be considered, it must be admitted that in the present status of our knowledge, they do not very materially aid in the solution of the problem. The detection of such substances is even more difficult than that of the enzyme proper and the expectation of success on their identification or effects in the types of activity manifested by plants must be remote.

As to the enzymes themselves, experiment seems to afford some opportunity for securing information. The method by which evidence as to their presence or absence might be arrived at, is by making extracts of the organ in question, and injecting these into parts in which they are lacking. A series of experiments with this object in view has been started but no results ready for publication have as yet been obtained. There are certain inherent difficulties in manipulation, which make the success of the operation uncertain; but further work may suggest means of obviating these, or at least of minimizing their influence.

Such an explanation as the one above outlined would not necessarily preclude the action of any of the conditions that have been assigned as the causes of regeneration. It simply indicates that, as the regeneration is lacking in cases for which these theories fail to account, there may be necessary, in addition, specific substances which can be provisionally looked upon in the light of enzymes.

SUMMARY

1. Every organ of the plant without buds that has been used as a cutting has been found to be capable of a certain amount of regeneration.

2. In the cases of the roots that regenerated shoots, these were not confined to the basal ends of the cuttings, but were found on the apical cut-surface as well (horseradish) or upon the surface which at the time was under the sand (parsnip) or, finally, in the middle of the root (*Pelargonium*).

3. The separate tissue regions of the root exclusive of the cambium were found capable of regeneration. From the central part of the horseradish, shoots were developed, and both the rind and the central cylinder of the parsnip regenerated, though the latter produced only roots.

4. Parts of six other kinds of roots were able to form roots, either as regenerations or as outgrowths through the rind in the normal way, but did not develop shoots.

5. When the bud-producing regions were cut away from a number of stems, these formed roots from the base, but were unable to replace the shoots. Though such parts lived over a period in which, on the plant, they would alter their form and become secondarily thickened, they did not undergo any change when treated in the manner described. In the *Muhlenbeckia*, however, when a single bud was left, such thickening, though of an unusual sort, did occur.

6. If in the shoots of the potato used as cuttings, one bud was left uninjured anywhere on the stem, this developed into an aërial tuber; if all buds were removed, no shoot-formation took place.

7. Budless parts of potato tubers were found capable of forming new shoots on one of the cut surfaces of the nodes.

8. Regeneration of both kinds of organs occurred at the base of the pseudo-bulb of *Dendrobium Parishii.*

9. The production of a single root from stems of the seedlings of *Pinus Laricio* and also from one of the older parts shows that this kind of regeneration externally resembling a "restoration" may take place from stems as well as from the apical ends of roots.

10. The majority of leaves used as cuttings regenerated roots only; but two new cases were found (*Piper canescens*) and the potato-leaf (*Solanum tuberosum*) which were able to form a shoot and thus reproduce the plant. The roots of such leaves can arise either from a callus or directly from the tissue of the petiole. Modified leaves such as phyllodes and bulb scales were also able to form roots.

11. Leaves which on the plant have a very short existence were found able to prolong this considerably when they formed roots.

12. Regeneration of both kinds of organs occurred in the flower stalks of *Dudleya californica*, and of roots in the inflorescences of *Bryophyllum calycinum* and *Ruellia rosea.* The fruits of *Phaseolus vulgaris* and *P. lunatus* also formed calluses and roots.

13. The disposition to form roots is much more generalized throughout the plant and more easily energized than the power to form shoots. Only about one fourth of the parts used produced shoots in addition to roots, and only three produced shoots alone. This power cannot be explained on structural or teleological grounds.

14. The alga *Penicillus capitatus* has the power of replacing the "head" of filaments when this is cut off.

15. Experiments show that regeneration is dependent upon an adequate food-supply. In plants from which the reserve food had been exhausted by a prolonged exclusion from the light, no regeneration took place if the parts were subsequently darkened,

or if the carbon dioxide was absorbed from the atmosphere in which the parts were kept. Entirely white shoots of several species of plants — presumably from their inability to manufacture food — proved unable to form roots even though primordia might be present.

16. Since certain parts fail to regenerate one or the other kind of organs, when each of the conditions previously assigned as the causes of regeneration has been fulfilled, it seems necessary to postulate the existence of specific substances which are responsible for the formation of those organs. These substances have been assumed to take the form of different enzymes which are not present in all the cells of the plant, but are localized in definite places. It is perhaps possible to obtain evidence on the grounds of this assumption by experiments, of which a series has been already started.

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